

# Role of active and passive recovery in adaptations to high intensity training

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## Abstract

It has been established that Wingate-based high-intensity training (HIT) consisting of 4 to 6 x 30-s all-out sprints interspersed with 4-min recovery is an effective training paradigm. Despite the increased utilisation of Wingate-based HIT to bring about training adaptations, the majority of previous studies have been conducted over a relatively short timeframe (2 to 6 weeks). However, activity during recovery period, intervention duration or sprint length have been overlooked. In study 1, the dose response of recovery intensity on performance during typical Wingate-based HIT (4 x 30-s cycle all-out sprints separated by 4-min recovery) was examined and active recovery (cycling at 20 to 40% of  $\dot{V}O_{2peak}$ ) has been shown to improve sprint performance with successive sprints by 6 to 12% compared to passive recovery (remained still), while increasing aerobic contribution to sprint performance by ~15%. In the following study, 5 to 7% greater endurance performance adaptations were achieved with active recovery (40%  $\dot{V}O_{2peak}$ ) following 2 weeks of Wingate-based HIT. In the final study, shorter sprint protocol (4 to 6 x 15-s sprints interspersed with 2 min of recovery) has been shown to be as effective as typical 30-s Wingate-based HIT in improving cardiorespiratory function and endurance performance over 9 weeks with the improvements in  $\dot{V}O_{2peak}$  being completed within 3 weeks, whereas exercise capacity (time to exhaustion) being increased throughout 9 weeks. In conclusion, the studies demonstrate that active recovery at 40%  $\dot{V}O_{2peak}$  significantly enhances endurance adaptations to HIT. Further, the duration of the sprint does not seem to be a driving factor in the magnitude of change with 15 sec sprints providing similar adaptations to 30 sec sprints. Taken together, this suggests that the arrangement of recovery mode should be considered to ensure maximal adaptation to HIT, and the practicality of the training would be enhanced via the reduction in sprint duration without diminishing overall training adaptations.

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## **List of Abbreviations**

ADP Adenosine diphosphate

AMP Adenosine monophosphate

AMPK AMP-activated protein kinase

ANOVA Analysis of variance

ATP Adenosine triphosphate

a-  $\bar{v}$  O<sub>2</sub> difference Arterial-mixed venous oxygen difference

BIA Bioelectrical impedance analysis

Ca<sup>2+</sup> Calcium ion

CaMK Calcium/calmodulin-dependent protein kinase

CHO Carbohydrate

COP Crossover point

COX Cytochrome *c* oxidase

CO<sub>2</sub> Carbon dioxide

CP Critical power

Cr Creatine

CS Citrate synthase

DBP Diastolic blood pressure

DEXA Dual-energy X-ray absorptiometry



FAD Flavin adenine dinucleotide

FADH<sub>2</sub> Reduced form of FAD

FAT/CD 36 Fatty acid translocase

FABPpm Plasma membrane-associated fatty acid binding protein

FFM Free fat mass

GDP Guanosine diphosphate

GTP Guanosine triphosphate

H<sup>+</sup> Hydrogen ion

HIT High-intensity training

HR Heart rate

HRmax Heart rate max

IMP Inosine monophosphate

LBM Lean body mass

LDH Lactate dehydrogenase

LSD test Least significant difference test

MAP Mean arterial pressure

MAPK Mitogen-activated protein kinase

MAV Maximal aerobic velocity

MCT Monocarboxylate transporter

MRI Magnetic resonance imaging

mRNA Messenger RNA

MRS Magnetic resonance spectroscopy

NAD<sup>+</sup> Nicotinamide adenine dinucleotide

NADH Reduced form of NAD<sup>+</sup>

NH<sub>4</sub><sup>+</sup> Ammonia

NIRS Near infrared spectroscopy

OCP Oral contraceptive pill

PARQ Physical activity readiness questionnaire

PCr Phosphocreatine

PGC-1α Peroxisome proliferator-activated receptor γ co-activator 1α

PDH Pyruvate dehydrogenase

PFK Phosphofructokinase

pH A measure of acidity/alkalinity.  $\text{pH} = -\log_{10}[\text{H}^+]$

PHOS Glycogen phosphorylase

P<sub>i</sub> Inorganic phosphate

P<sub>max</sub> maximal power output

RER Respiratory exchange ratio

RNA Ribonucleic acid

ROS Reactive oxygen species

RPM Revolution per minute

SBP Systolic blood pressure

SD Standard deviation

SV Stroke volume

TBM Total body mass

TCA cycle Tricarboxylic acid cycle

TOI Tissue oxygen index

TTE Time to exhaustion

T to 50 $\dot{V}O_{2peak}$  Time required to reach 50% of  $\dot{V}O_{2peak}$

$\dot{V}CO_2$  Carbon dioxide production

$\dot{V}O_2$  Oxygen uptake

$\dot{V}O_{2max}$  Maximal oxygen uptake

$\dot{V}O_{2peak}$  Peak oxygen uptake

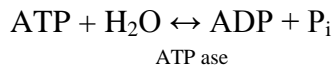


# **Chapter 1**

## **General Introduction**

## 1.1 Energy metabolism

Regardless of exercise mode or activity level, human bodies derive energy for activity from the hydrolysis of adenosine triphosphate (ATP) via the enzyme ATPase.



*Equation 1.1: ATP utilisation during muscular contraction, where ADP is adenosine diphosphate and  $\text{P}_i$  is inorganic phosphate.*

The storage of intramuscular ATP has been reported to be 20 to 25 mmol·kg dry muscle<sup>-1</sup> (Glaister 2005; Spencer et al. 2005), and the concentration of ATP is normally well preserved since it is re-synthesised from ADP at the same rate at which it is utilised in most situations (Maughan and Gleeson 2010). During intense exercise (e.g. repeated maximal sprints), however, the concentration of ATP in type II muscle fibres has been shown to be decreased by ~ 50 to 60% from pre-exercise values, whereas that in type I fibres has been found to be depleted only modestly (15 to 20%) (Esbjornsson-Liljedahl et al. 1999, 2002). Nevertheless, this suggests that the regulatory mechanisms do not seem to allow its complete depletion even when the demand for ATP is at its highest (Esbjornsson-Liljedahl et al. 1999, 2002; Gastin 2001; Glaister 2005). Furthermore, intramuscular ATP has been suggested to fuel only about 2 seconds of maximal work (Glaister 2005; Maughan and Gleeson 2010; McArdle et al. 2010), and thus ATP has to be re-synthesised very quickly when a maximal effort is required. Where transient decrease in ATP and increases in ADP and  $\text{P}_i$  occur such as at the onset of muscular contraction or during intense exercise, enzymes related to the breakdown of other intramuscular fuel stores are activated to re-synthesise ATP (Maughan and Gleeson 2010; McArdle et al. 2010). ATP is produced with or without the utilisation of oxygen in skeletal muscle depending on the intensity and duration of exercise being performed although there is

always an interaction between different metabolic pathways (Gastin 2001; Maughan and Gleeson 2010).

### *1.1.1 Aerobic metabolism (oxidative phosphorylation)*

The regeneration of ATP is mainly supported by aerobic metabolism when performing steady-state exercise with an intensity up to lactate or pulmonary gas exchange threshold (Jones and Burnley 2009). The term *aerobic metabolism* (or *oxidative phosphorylation*) indicates that ATP is derived from the breakdown of stored carbohydrate (muscle glycogen or blood glucose) and fat with the use of oxygen in the mitochondria of skeletal muscle (Hargreaves 2006; Horowitz 2006; Figure 1.1). The major advantage of aerobic metabolism is that it can provide ATP for several hours during light to moderate-intensity exercise (e.g. walking, jogging) where the demand for ATP is well met by oxidative ATP provision (Spriet 2006). However, it requires a finite time for the aerobic energy system to reach its maximal rate of ATP provision and oxidative phosphorylation is not able to provide sufficient ATP during intense exercise where ATP demand exceeds the maximal rate of oxidative ATP provision (i.e.  $> \dot{V}O_{2\max}$ ) (Spriet 2006). Therefore, there is a gap between ATP demand and provision at the onset of exercise or during intense exercise and it needs to be filled via different energy pathways.

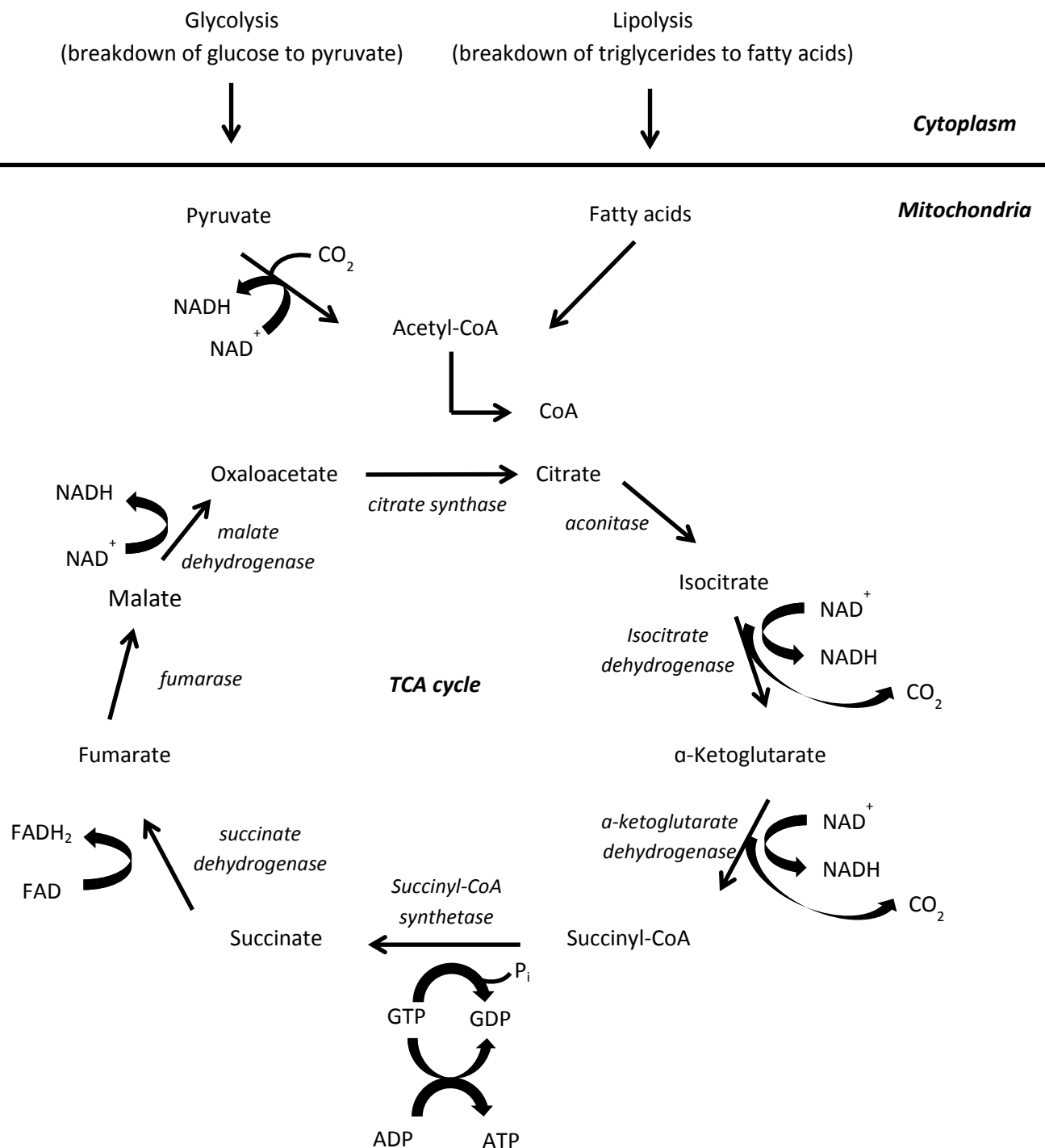


Figure 1.1: An overview of oxidative phosphorylation (Powers and Howley 2009)

### 1.1.2 Anaerobic metabolism (substrate phosphorylation)

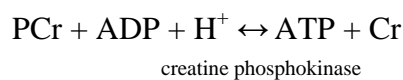
During intense exercise or at the onset of exercise, the re-synthesis of ATP is chiefly derived from the breakdown of muscle phosphocreatine and glycogen. Since these processes take place without the immediate use of oxygen, it is called anaerobic metabolism or substrate



phosphorylation (Spriet 2006). It has been shown that the utilisation of PCr and glycogen occurs simultaneously during the commencement of maximal or near maximal exercise (Spencer et al. 2005).

#### *1.1.2.1 Phosphocreatine*

The most immediate energy reserve for ATP re-synthesis at the onset of muscular contraction is phosphocreatine (PCr). PCr breakdown is catalysed by the enzyme creatine phosphokinase.



*Equation 1.2: PCr conversion to ATP, where  $\text{H}^+$  is hydrogen ion and Cr is free creatine.*

Human muscles store approximately  $80 \text{ mmol} \cdot \text{kg dry muscle}^{-1}$  of phosphocreatine (Maughan and Gleeson 2004; Glaister 2005). Since the hydrolysis of PCr does not require oxygen, it reaches its maximal rate of ATP provision (approximately  $9.0 \text{ mmol ATP} \cdot \text{kg dry muscle}^{-1} \cdot \text{sec}^{-1}$ ) within the first 1-2sec of contraction (Maughan and Gleeson 2004; Glaister 2005). During maximal exercise, however, PCr stores are largely depleted within the initial 10 sec following the onset of muscular contraction (Bogdanis et al. 1996a; Bogdanis et al. 1998).

#### *1.1.2.2 Anaerobic glycolysis*

Another immediate reserve for ATP re-synthesis is stored carbohydrate, mainly in the form of muscle glycogen. This process involves glycogenolysis (the hydrolysis of muscle glycogen to glucose 1-phosphate) and glycolysis (the breakdown of glucose to pyruvate). The term *anaerobic glycolysis* indicates that this process takes place in the absence of oxygen and results in lactate formation (Figure 1.2).

Although the storage of glycogen is superior to that of PCr (approximately  $300 \text{ mmol} \cdot \text{kg dry muscle}^{-1}$ ; Maughan and Gleeson 2004; Glaister 2005), it takes around 5 to 10 sec to reach its

maximal rate for ATP production (Parolin et al. 1999; Glaister 2005) and the maximal rate of ATP re-synthesis from glycolysis is approximately half of that derived from the degradation of PCr ( $4.5 \text{ ATP} \cdot \text{kg dry muscle}^{-1} \cdot \text{sec}^{-1}$ ) (Maughan and Gleeson 2004). This is because that glycolytic pathway involves series of reactions and requires several key enzymes catalysing its process such as glycogen phosphorylase (PHOS; catalyses the breakdown of stored muscle glycogen to glucose 1-phosphate), phosphofructokinase (PFK; catalyses the phosphorylation of the glycolytic intermediate fructose 6-phosphate) and lactate dehydrogenase (LDH; catalyses the conversion of pyruvate to lactate) (Ross and Leveritt 2001; Maughan and Gleeson 2004; Spriet 2006; Figure 1.2).

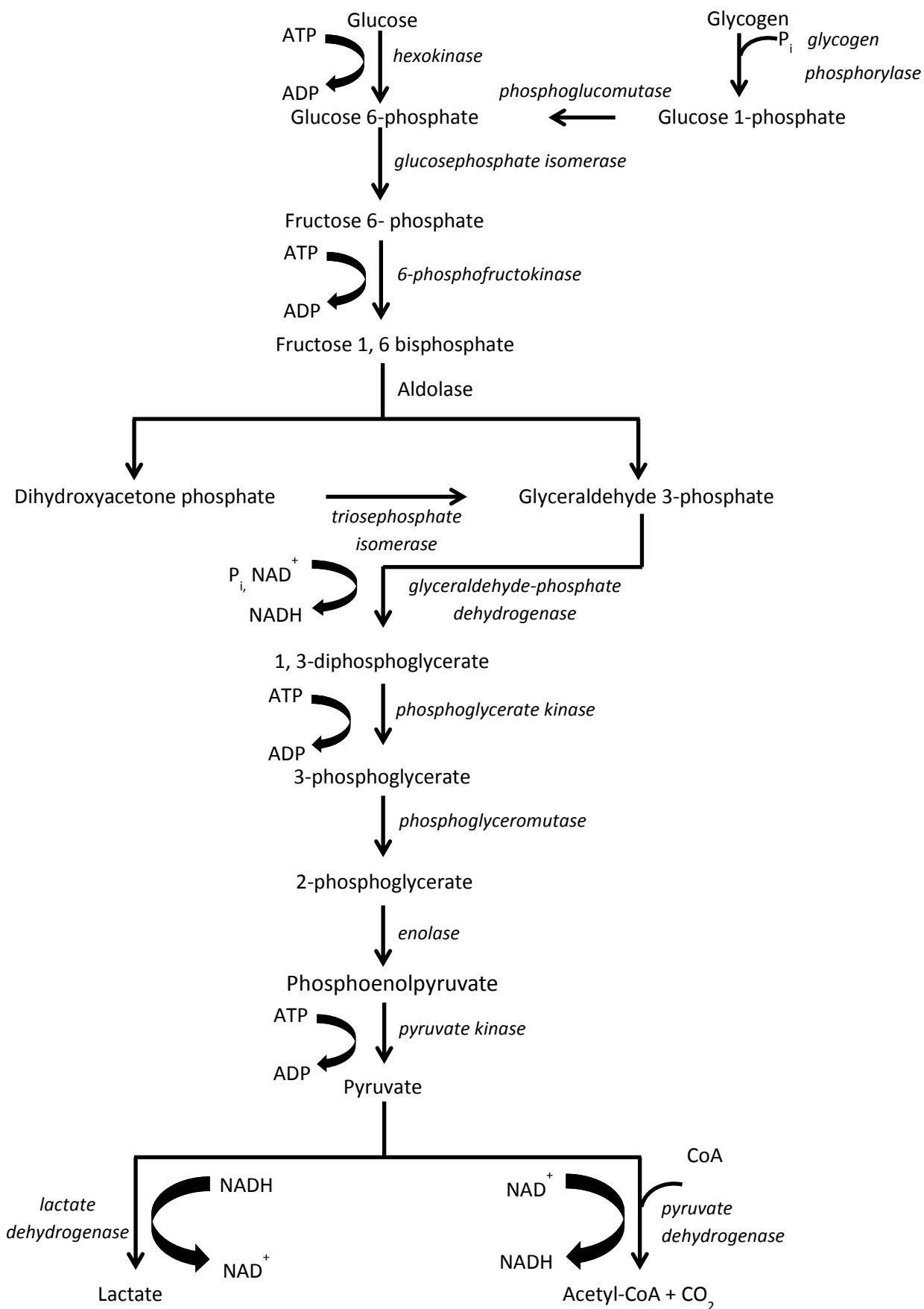
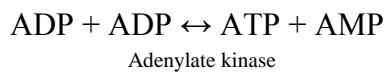


Figure 1.2: The glycolytic pathway (Maughan and Gleeson 2004)

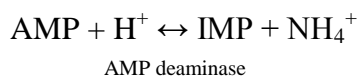
### *1.1.2.3 Adenylate kinase reaction*

During extremely intense exercise such as all-out sprinting, a small amount of ATP can be derived from pairs of ADP molecules (Glaister 2005; Spriet 2006). This reaction is catalysed via enzyme adenylate kinase and results in the formation of ATP and adenosine monophosphate (AMP) (Eq 1.3; Glaister 2005; Spriet 2006).



### *Equation 1.3: Adenylate kinase reaction*

The generated AMP is further deaminated to inosine monophosphate (IMP) and ammonia ( $\text{NH}_4^+$ ) via enzyme AMP deaminase.



### *Equation 1.5*

These reactions may temporarily reduce the concentration of adenine nucleotides, however, once muscular contraction has stopped, the majority are re-synthesised via the purine nucleotide cycle (Glaister 2005; Spriet 2006).

## **1.2 Exercise metabolism during maximal exercise**

Energy provision during a single maximal intensity bout such as the Wingate test draws energy from a number of different pathways (Figure 1.3).

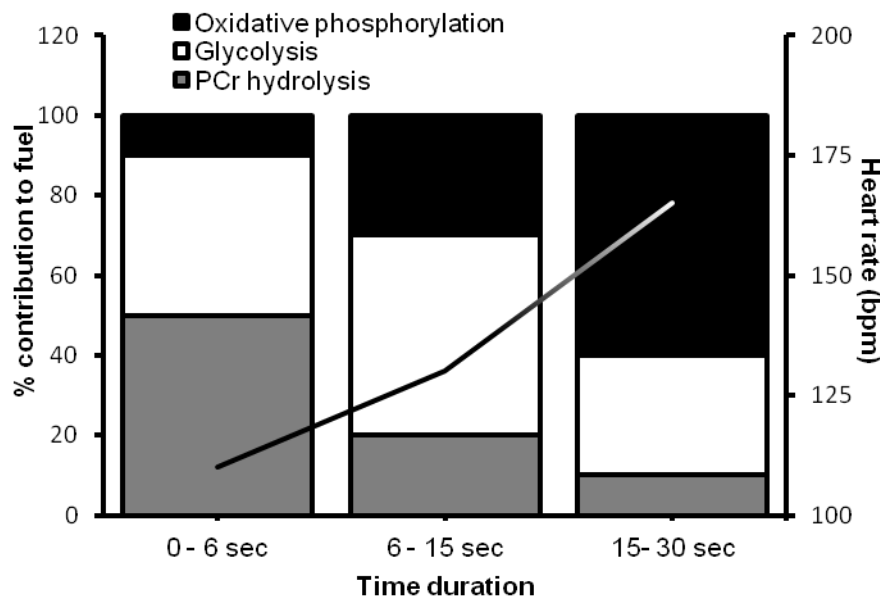


Figure 1.3: Different fuel contributions to a single Wingate test (Parolin et al. 1999)

Although there is a small contribution from oxidative metabolism (carbohydrate oxidation) ( $\leq 10\%$ ) even in the first 6 seconds, ATP is mainly derived from anaerobic metabolism or substrate phosphorylation (Gastin 2001; Maughan and Gleeson 2004). After 6 seconds the energy provision is predominantly from anaerobic glycolysis with an increasing oxidative load (Parolin et al. 1999; Figure 1.3). In the final 15 seconds of a Wingate test, oxidative metabolism meets the largest portion of energy demand, with a smaller contribution of anaerobic glycolysis and PCr hydrolysis (Parolin et al. 1999; Figure 1.3). In terms of power production during the Wingate test the maximum power is reached within 2-3 sec and is much greater than is possible to achieve through aerobic metabolism (typically 300-400% of  $\dot{V}O_{2max}$ ) (Maughan and Gleeson 2004; Spriet 2006), due to the predominate utilisation of PCr (Figure 1.3). However, such high power output can be sustained for only a short period of time since the rate of PCr degradation begins to drop after only a few seconds (Gastin 2001; Maughan and Gleeson 2004). Further, this energy source is largely depleted within 10 sec when maximal effort is required (Bogdanis et al. 1996a; Bogdanis et al. 1998). Whilst ATP

re-synthesis from anaerobic glycolysis reaches its maximal rate after 5 sec, it can only be maintained at this high rate for several seconds (Parolin et al. 1999; Maughan and Gleeson 2004; Figure 1.3). Indeed, it has been demonstrated that glycolysis is severely attenuated during the second 15 sec of a 30-s maximal exercise (Parolin et al. 1999; Figure 1.3). This reduction in anaerobic metabolism results in a sustained drop in power output during the Wingate test (Figure 1.4).

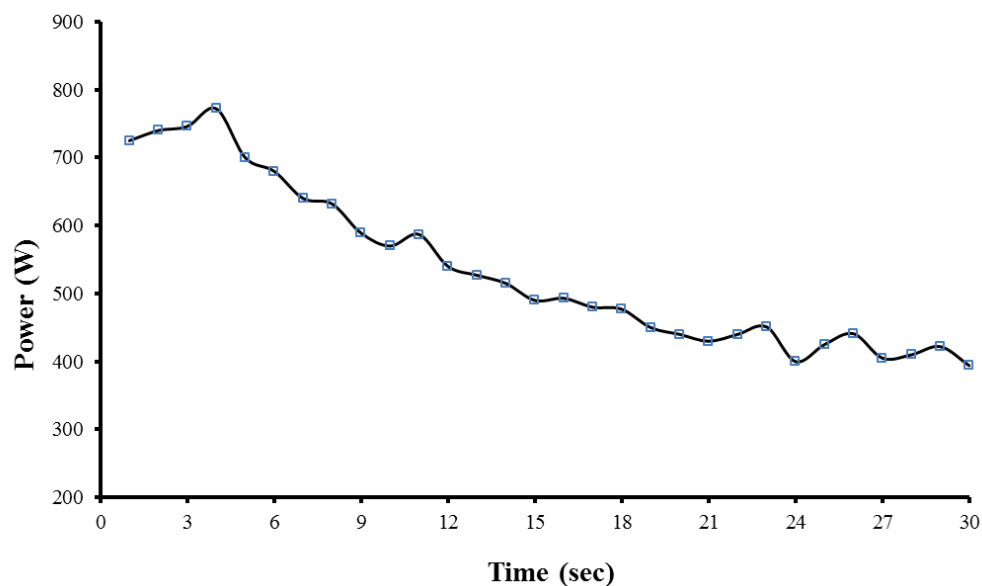


Figure 1.4 Typical power profile across a 30 second Wingate test

As the sprint progresses the energy is increasingly being generated by aerobic metabolism with a subsequent loss of power production (Bangsbo et al. 1990; Figure 1.3 & 1.4).

Furthermore, the importance of aerobic contribution to performance has been shown to increase with repeated bouts of intense exercise.

### 1.3 Metabolic responses to repeated sprint exercise

It has been shown that when maximal exercise is interspersed with insufficient recovery periods, the contribution from oxidative phosphorylation to performance increases with

successive bouts due to a large decrease in anaerobic ATP provision. Gaitanos et al. (1993) employed ten 6-s maximal cycle sprints interspersed with 30 sec of passive recovery, and estimated anaerobic ATP provision from the changes in muscle metabolites during the first and last 6-s sprints. There was a similar contribution from PCr degradation and anaerobic glycolysis to anaerobic ATP production during the first sprint (50 and 44%, respectively). However, the contributions from PCr degradation and glycolysis to the provision of ATP were noticeably changed during the last sprint with the former being increased to 80% and the latter being reduced to 16%. Moreover, whilst total anaerobic ATP production was decreased by 65% from sprint 1 to 10, mean power output generated in the last sprint was only reduced by 27% compared with that produced in the first sprint, suggesting that power produced during the last sprint was supported by energy mainly derived from PCr degradation and an increased aerobic metabolism (Gaitanos et al. 1993).

In addition, Bogdanis et al. (1996a) investigated the contribution of anaerobic and aerobic metabolism during 2 x 30-s maximal cycling sprints separated by 4 min of passive recovery (i.e. Wingate test), and found approximately a 41% decrease in anaerobic metabolism during the second sprint mainly due to a ~ 45% reduction in glycolysis. During sprint 2, the initial 10-s power was mainly supported by PCr degradation with the majority of energy demand being met by an increased oxidative metabolism for the rest of the sprint (Bogdanis et al. 1996a). Indeed, almost a half of energy (approximately 49%) was derived from aerobic metabolism during the second sprint, and consequently, total work produced during sprint 2 was only reduced by 18% despite the large decrease (41%) in anaerobic energy release (Bogdanis et al. 1996a). Likewise, Parolin et al. (1999) have indicated that the contribution from anaerobic glycolysis to 30-s sprint performance is negligible from sprint 3 onwards using 3 x 30-s maximal isokinetic cycling separated by 4-min recovery. They found a marked

decrease in muscle glycogen utilisation (by ~ 95%) from sprint 1 to 3. Conversely, PCr utilisation was only reduced by 26% and there was a 10% increase in the rate of ATP supply from oxidative phosphorylation from sprint 1 to 3, resulting in only a 26% power drop relative to sprint 1 (Parolin et al. 1999). These findings suggest that there is an increased reliance upon aerobic metabolism with successive sprint repetitions due to a reduced contribution from anaerobic metabolism, especially glycolysis.

#### **1.4 Oxygen uptake kinetics during and following exercise**

It has been suggested that oxygen uptake response during exercise ( $\dot{V}O_2$  on-kinetics) is an important factor in determining exercise performance (Burnley and Jones 2007; Jones and Burnley 2009). Whilst oxygen uptake rises exponentially regardless of exercise intensity following the onset of dynamic exercise, the time course and amplitude of  $\dot{V}O_2$  response are determined by ATP demand of exercise performed (Stirling et al. 2005; Burnley and Jones 2007; Jones and Burnley 2009; Figure 1.5). During low- to moderate-intensity exercise below the lactate or pulmonary gas exchange threshold, oxygen uptake reaches its steady-state level within 2 to 3 min (Jones and Burnley 2009; Chidnok 2012; Figure 1.5). Above this exercise intensity; however, the initial exponential  $\dot{V}O_2$  response is followed by a delayed onset of rise in  $\dot{V}O_2$  (so-called “ $\dot{V}O_2$  slow component”), which is mostly attributed to an increased rate of type II muscle fibre recruitment with increasing exercise intensity (Burnley and Jones 2007; Jones and Burnley 2009). During heavy-intensity exercise typically represented by the critical power, it results in a delayed and elevated steady-state  $\dot{V}O_2$ , whereas it continues to rise until maximal oxygen uptake is achieved above the critical power (severe-intensity) (Jones et al. 2010; Chidnok et al. 2012; Figure 1.5). The recovery kinetics of oxygen uptake ( $\dot{V}O_2$  off-kinetics) is also dependent on exercise intensity. It has been shown that  $\dot{V}O_2$  declines in a mono-exponential manner following moderate to heavy-intensity exercise, whereas the early



exponential  $\dot{V}O_2$  decline is supplemented by a delayed onset of  $\dot{V}O_2$  drop when preceded by severe-intensity exercise (Özyener et al. 2001; Perrey et al. 2002). Similar time constants of the fast component of  $\dot{V}O_2$  recovery (30 to 40 sec), on the other hand, have been reported regardless the preceding exercise intensity (Özyener et al. 2001; Perrey et al. 2002; Dupont et al. 2010; Buchheit et al. 2012), which is typically completed within 2 to 3 min (Perrey et al. 2002). The rapid component of excess oxygen consumption during recovery has been attributed to the replenishment of intramuscular PCr as well as oxygen stores in blood and muscle (Perrey et al. 2002; Børsheim and Bar 2003). In addition, it has been established that the recovery of high-intensity exercise performance is largely determined by the magnitude of PCr restoration (Bogdanis et al. 1995, 1996a; Mendez-Villanueva et al. 2012). Since the ATP derived from oxidative metabolism is used for the re-synthesis of PCr following exercise (Harris et al. 1976; Haseler et al. 1999), oxygen availability has been suggested to be important for PCr recovery (Haseler et al. 1999). Indeed, the time course of  $\dot{V}O_2$  recovery has been shown to reflect that of PCr restoration (Cohen-Solal et al. 1995; McMahon and Jenkins 2002). Taken together, while an accelerated  $\dot{V}O_2$  adjustment in response to exercise would result in an increased exercise tolerance by delaying the onset of depletion of high-energy phosphates and accumulation of fatigue-related metabolites (e.g.  $H^+$ ,  $P_i$ ) (Jones et al. 2008; Jones and Burnley 2009; Vanhatalo et al. 2010),  $\dot{V}O_2$  off-kinetics would play a role in determining exercise performance where high-intensity bouts are repeated interspersed with insufficient recovery (Tomlin and Wenger 2001; Dupont et al. 2010).

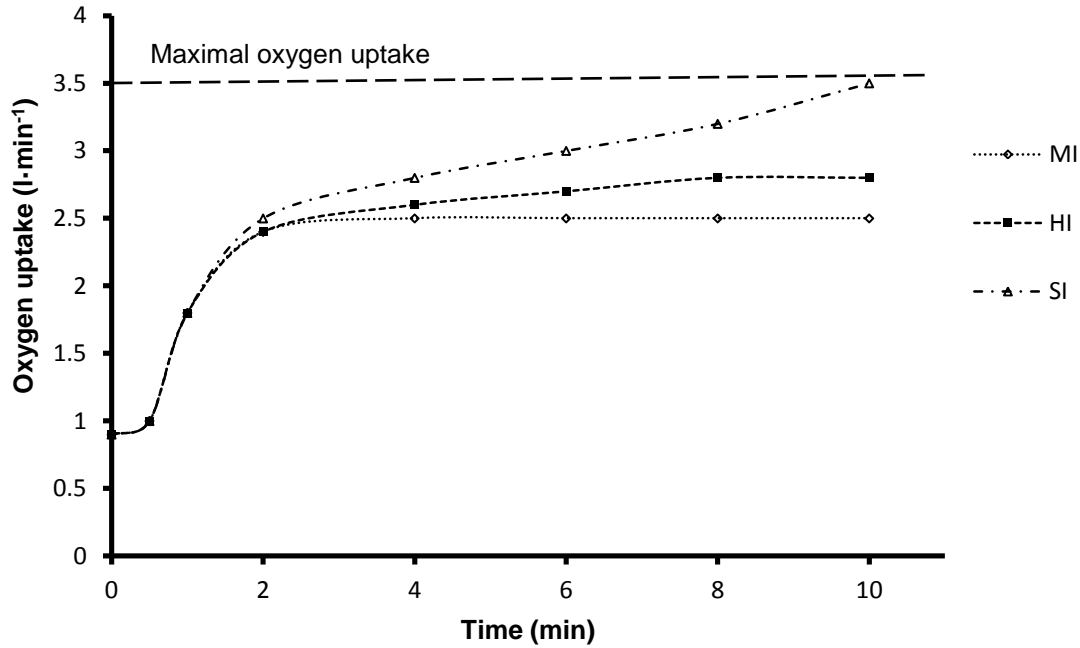


Figure 1.5: Example of oxygen uptake responses to exercise at different intensities (Burnley and Jones 2007). MI, moderate-intensity; HI, heavy-intensity; SI, severe-intensity

### 1. 5 Relationships between aerobic metabolism and repeated sprint performance

Given an increased contribution of aerobic metabolism to performance with successive sprint bouts, aerobic capacity may have an impact on repeated sprint performance. Hamilton et al. (1991) investigated differences in physiological responses to maximal intermittent exercise between endurance-trained runners (ET,  $\dot{V}O_{2max}$ :  $60.8 \pm 4.1 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) and team players (TP,  $\dot{V}O_{2max}$ :  $52.4 \pm 4.9 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) using ten 6-s sprint on a non-motorized treadmill interspersed with 24-s rest periods. In their study, the ET group consumed more oxygen than the TP group (ET vs. TP:  $35.0 \pm 2.2$  vs.  $29.6 \pm 3.0 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ,  $P < 0.05$ ), and showed significantly smaller decline in mean power output over the 10 repetitive sprints (ET vs. TP:  $14.2 \pm 11.1\%$  vs.  $29.3 \pm 8.1\%$ ,  $P < 0.05$ ). However, the higher power drop rate seen in the TP might be related to their non-significant but greater peak power during the first sprint (TP vs. ET:  $839 \pm 114$  vs.  $777 \pm 89 \text{ W}$ ) since a strong correlation has been shown between initial power production and performance decrement during repeated sprint exercise (Bogdanis et al.

1995; Edge et al. 2006; Girard et al. 2011). To address this issue, Bishop and Edge (2006) investigated the relationship between  $\dot{V}O_{2\max}$  and repeated sprint performance (5 x 6-s all-out sprints every 30 sec), while controlling the effects of initial sprint performance on sprint decrement. Their female subjects were assigned to either moderately-trained (MT) or untrained (UT) group according to their fitness level as assessed by  $\dot{V}O_{2\max}$  (MT vs. UT:  $49.6 \pm 4.8$  vs.  $36.4 \pm 4.7$  ml·kg<sup>-1</sup>·min<sup>-1</sup>). While there was no significant difference between the groups for peak power output (MT vs. UT:  $835 \pm 127$  W vs.  $788 \pm 99$  W) or total work (MT vs. UT:  $3.58 \pm 0.49$  kJ vs.  $3.44 \pm 0.57$  kJ) in the initial sprint, the MT group showed a smaller work decrement across the five sprints (MT vs. UT:  $7.6 \pm 3.4$  vs.  $11.1 \pm 2.5\%$ ,  $P < 0.05$ ). Considering that there was no difference in other factors such as muscle buffer capacity or metabolic responses (muscle lactate and pH) between the groups, the greater aerobic capacity seen in the MT group seems to have induced the smaller work decrement. Likewise, Tomlin and Wenger (2002) examined the relationships between aerobic fitness, power maintenance and oxygen consumption using a similar repeated sprint protocol (ten 6-s cycle sprints alternated with 30 sec of recovery), and greater oxygen uptake was seen in moderate aerobic power group (MOD,  $\dot{V}O_{2\max}$ :  $47.6 \pm 3.8$  ml·kg<sup>-1</sup>·min<sup>-1</sup>) compared with lower aerobic power group (LOW,  $\dot{V}O_{2\max}$ :  $34.4 \pm 2.4$  ml·kg<sup>-1</sup>·min<sup>-1</sup>) in 9 of 10 sprint-recovery cycles. Whilst both groups produced similar power over the first six sprints, the MOD group maintained better power during each of the last four sprints, resulting in a small power drop over the 10 sprints (MOD vs. LOW:  $8.8 \pm 3.7$  vs.  $18.0 \pm 7.6\%$ ,  $P < 0.05$ ) (Tomlin and Wenger 2002).

Furthermore, Hamilton et al. (1991) observed a greater blood lactate accumulation in the team players compared to the endurance-trained runners following the 10 sprints (TP vs. ET:  $15.2 \pm 1.9$  vs.  $12.4 \pm 1.7$  mmol·l<sup>-1</sup>), and there was a strong correlation between peak blood lactate and power drop rate ( $r = 0.92$ ). This led to the conclusion by the authors that the

greater power decline seen in the TP may be associated with their higher glycolytic rate and lower oxygen uptake. Similarly, Balsom et al. (1994) demonstrated that reduced oxygen availability (via hypoxic condition) resulted in higher blood lactate accumulation, lower oxygen uptake and impaired performance during repeated cycling sprints (10 x 6-s sprint interspersed with 30 sec of recovery). Nonetheless, since the level of blood lactate can only indicate the balance between muscle lactate production and its consumption by several tissues (e.g. heart, liver and skeletal muscles) (Brooks 2000; Gladden 2000), which is mediated by various factors such as blood flow, lactate concentration, hydrogen ion concentration and membrane lactate transporters (monocarboxylate transporters, MCTs) (Gladden 2000), blood lactate accumulation itself does not necessarily provide an accurate estimation of the level of lactate in the working muscle (Rodas et al. 2000). Moreover, although a high level of blood lactate is often associated with performance decline in repeated sprint exercise (Hamilton et al. 1991; Balsom et al. 1994), it may simply reflect a high rate of anaerobic glycolysis during the initial sprints, especially in individuals with high anaerobic capacity (Ross and Leveritt 2001), which typically results in attenuated glycogen metabolism and thus power decline during the latter sprints (Gaitanos et al. 1993; Bogdanis et al. 1996a; Parolin et al. 1999). Indeed, previous studies showed a disassociation between repeated sprint performance and blood lactate accumulation (Bogdanis et al. 1996b; Connolly et al. 2003) and the importance of acidosis in repeated sprint performance has been questioned (Glaister 2005). Therefore, muscle or blood lactate level itself might not explain changes in performance during repeated sprint exercise. Instead, an enhanced aerobic response (e.g. faster oxygen kinetics) may become increasingly important for performance with successive bouts (Dorado et al. 2004) where anaerobic metabolism progressively decreases (Gaitanos et al. 1993; Bogdanis et al. 1996a; Parolin et al. 1999). Dupont et al. (2005) found positive relationships between  $\dot{V}O_2$  on-kinetics during constant load exercise and speed decrement during repeated sprint exercise

(15 x 40-m sprints separated by 25-s recovery) ( $r = 0.8$ ) and cumulated time for the 15 sprints ( $r = 0.8$ ). Moreover, faster  $\dot{V}O_2$  off-kinetics following severe-intensity exercise has been associated with a smaller speed drop over seven 30-m sprints alternated with 20 sec of recovery ( $r = 0.85$ ) (Dupont et al. 2010). As mentioned in the previous section, this indicates that an accelerated recovery of  $\dot{V}O_2$  following exercise may reflect an enhanced PCr restoration (Cohen-Solal et al. 1995; Tomlin and Wenger 2001; McMahon and Jenkins 2002). In short, it seems that greater oxygen uptake or faster  $\dot{V}O_2$  kinetics would become increasingly important with successive bouts, while faster  $\dot{V}O_2$  off-kinetics between high-intensity bouts may facilitate the re-synthesis of phosphocreatine. Thus, recovery intensity during repeated sprint exercise may influence overall performance due to an alteration in aerobic metabolism.

#### **1.6 Effects of recovery mode on repeated sprint performance: Active or Passive?**

Studies to date indicate that when performing repeated sprint exercise, a choice of recovery mode should be made according to recovery duration (Table 1.1). When recovery duration is brief (15 to 21sec) relative to sprint duration (e.g. sprint: rest ratio between 1:1 to 1:5), active recovery has been shown to decrease repeated sprint performance including greater performance decline in subsequent sprints (Spencer et al. 2006, 2008; Dupont et al. 2007; Buchheit et al. 2009) and shorter time to exhaustion (Dupont et al. 2003, 2004) compared to passive recovery irrespective of recovery intensity (20 to 50% of  $\dot{V}O_{2max}$ ) (Dupont et al. 2003, 2004, 2007; Spencer et al. 2006, 2008). Possible adverse effects associated with active recovery in these studies include a lower level of intramuscular phosphocreatine restoration or muscle re-oxygenation due to a greater oxygen cost induced by muscle activation during active recovery, suggesting that less oxygen is being available to re-synthesise

phosphocreatine and to reload haemoglobin and myoglobin (Dupont et al. 2003, 2004, 2007; Spencer et al. 2006, 2008; Buchheit et al. 2009).

Conversely, active recovery seems to enhance overall sprint performance when repeated efforts are interspersed with relatively long recovery period (180 to 240 sec; sprint: rest ratio between 1:8 to 1:12) (Bogdanis et al. 1996b; Connolly et al. 2003; Spierer et al. 2004). Connolly et al. (2003) employed repeated cycle sprints consisting of  $6 \times 15$ -s sprints alternated with 3-min recovery, and demonstrated that power out was better maintained during active recovery (cycling at 80W) compared to passive recovery. Similarly, other studies employing repeated 30-s Wingate tests (i.e. repeated 30-s sprints separated by 4 min of recovery) demonstrated that active recovery at light to moderate intensity (28 to 40%  $\dot{V}O_{2max}$ ) results in higher mean power output and greater total work compared with passive recovery (Bogdanis et al. 1996b; Spierer et al. 2004). With longer recovery, an elevated aerobic metabolism induced by active recovery (Bogdanis et al. 1996b; Dorado et al. 2004) may result in an improved repeated sprint performance. Bogdanis et al. (1996b) observed greater heart rate and  $\dot{V}O_2$  during the 4-min recovery period in active recovery condition (cycling at 40%  $\dot{V}O_{2max}$ ) compared to passive recovery, and the improved 30-s mean power output with the active recovery in sprint 2 was totally attributed to a 3.1% higher power output produced during the initial 10 sec. In another study, they also found a high correlation between re-synthesis of PCr and recovery of power output during the initial 10-s of the second 30-s sprint ( $r = 0.84$ ) (Bogdanis et al. 1996a). This suggests that increased blood flow to the previously worked muscle and systemic  $\dot{V}O_2$  induced by active recovery may allow greater oxygen availability to facilitate PCr restoration compared with passive recovery (Bogdanis et al. 1996b; Dorado et al. 2004; Dupont et al. 2007). Moreover, it has been shown that active recovery (20% of  $\dot{V}O_{2max}$ ) induces faster oxygen kinetics, greater aerobic

contribution to the total ATP provision, and consequently greater power production compared with passive recovery during intermittent supra-maximal-intensity (110% of  $\dot{V}O_{2\max}$ ) cycling (Dorado et al. 2004) separated by 5 min of recovery. Together, when performing repeated sprint exercise with a relatively long sprint-to-rest ratio (e.g. Wingate tests, sprint: rest ratio of 1:8), active recovery seems to induce an increased cardiorespiratory demand (e.g. higher  $\dot{V}O_2$  and HR) while enhancing overall sprint performance.

**Table 1.1 Summary of studies examining effects of recovery mode on repeated sprint performance**

Study	N	Exercise protocol	Recovery mode and duration	Performance measures	Results
Buchheit et al. (2009)	10M	6 x 4-s run sprints	AR ( $2.0\text{m}\cdot\text{s}^{-1}$ ) or PR; 21s	$\text{AvSp}_{\text{mean}}$ , $\text{Sp}\%$ $\text{Dec}$	PR > AR
Spencer et al. (2008)	9M	6 x 4-s cycle sprints	MIAR ( $35\% \dot{V}\text{O}_{2\text{max}}$ ), LIAR ( $20\% \dot{V}\text{O}_{2\text{max}}$ ) or PR; 21s	PPO in 2-6 sprints	PR > MIAR & LIAR
Spencer et al. (2006)	9M	6 x 4-s cycle sprints	AR ( $32\% \dot{V}\text{O}_{2\text{max}}$ ) or PR; 21s	$\text{PO}_{\text{Dec}}$	PR > AR
Dupont et al. (2007)	12M	15-s & 30-s cycle sprints	MIAR ( $40\% \dot{V}\text{O}_{2\text{max}}$ ), LIAR ( $20\% \dot{V}\text{O}_{2\text{max}}$ ) or PR; 15s	PPO, MPO	PR > MIAR & LIAR
Dupont et al. (2004)	12M	15-s cycling at $120\% \dot{V}\text{O}_{2\text{max}}$	AR ( $40\% \dot{V}\text{O}_{2\text{max}}$ ) or PR; 15s	TTE	PR > AR
Dupont et al. (2003)	12M	15-s run at $120\%$ MAS	AR ( $50\%$ MAS) or PR; 15s	TTE	PR > AR
Dorado et al. (2004)	10M	4 repeated cycling at $110\% \dot{V}\text{O}_{2\text{max}}$	AR ( $20\% \dot{V}\text{O}_{2\text{max}}$ ) or PR; 5min	TTE	AR > PR
Connolly et al. (2003)	7M	6 x 15s cycle sprints	AR ( $80\text{W}$ ) or PR; 3min	$\text{PO}_{\text{Dec}}$	AR > PR
Bogdanis et al. (1996b)	13M	2 x 30s cycle sprints	AR ( $40\% \dot{V}\text{O}_{2\text{max}}$ ) or PR; 4min	MPO	AR > PR
Spierer et al. (2004)	12M 3F	Serial 30s cycle sprints	AR ( $28\% \dot{V}\text{O}_{2\text{max}}$ ) or PR; 4min	MPO, TW	AR > PR

N, number of participants; M, male; F, female;  $\dot{V}\text{O}_{2\text{max}}$ , maximal oxygen uptake; MAS, maximal aerobic speed; AR, active recovery; PR, passive recovery; MIAR, moderate-intensity active recovery; LIAR, low-intensity active recovery;  $\text{AvSp}_{\text{mean}}$ , mean running speed;  $\text{Sp}\%$  $\text{Dec}$ , percentage speed decrement;  $\text{PO}_{\text{Dec}}$ , power output decrement; PPO, peak power output; MPO, mean power output; TTE, time to exhaustion; TW, total work



## **1.7 Influence of recovery mode on acute cardiorespiratory/cardiovascular responses and possible chronic adaptations induced by the arrangement of recovery modality**

In addition to heart rate and oxygen uptake, active recovery has been shown to increase stroke volume/cardiac output. Takahashi and Miyamoto (1998) demonstrated that stroke volume was greater with active recovery ( $\sim 28\% \dot{V}O_{2\max}$ ) compared to passive recovery ( $101.3 \pm 3.6$  vs.  $80.7 \pm 14.6$  ml,  $P < 0.05$ ) following short continuous cycling at 68% of  $\dot{V}O_{2\max}$ . Moreover, Crisafulli et al. (2004) demonstrated that active recovery (pedalling at 40W) increased cardiac output and reduced systemic vascular resistance following repeated cycling sprints compared to passive recovery. Likewise, Bogdanis et al. (1996b) observed similar mean blood pressure between active and passive recovery despite a significantly higher heart rate in the active recovery condition when two 30-s Wingate tests were separated by 4-min recovery. These findings suggest greater muscle blood flow and venous return with active recovery and a greater peripheral blood resistance with passive recovery when performing repeated sprints. In addition, it has been shown that SV reaches its peak value during a post-exercise period in a well-trained cyclist ( $\dot{V}O_{2\max}$ :  $69 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) in repeated sprint exercise consisting of 4 x 15-s sprints interspersed with 45 sec of passive recovery (Buchheit and Laursen 2013). Furthermore, Fontana et al. (2011) showed a similar cardiac demand between a 30-s Wingate test and a graded exercise test to exhaustion (i.e.  $\dot{V}O_{2\max}$  test) in healthy male participants ( $26.7 \pm 5.6$  years,  $\dot{V}O_{2\max}$ :  $45.0 \pm 5.3 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ). Whilst heart rate at the end of the Wingate test was lower than that observed at exhaustion in the graded test ( $149 \pm 26$  vs.  $190 \pm 12 \text{ beats}\cdot\text{min}^{-1}$ ,  $P < 0.001$ ), a greater stroke volume was induced by the Wingate test compared to the graded test ( $127 \pm 37$  vs.  $94 \pm 15$  ml,  $p < 0.001$ ), resulting in similar cardiac output (Wingate vs. Graded exercise test:  $18.2 \pm 3.3$  vs.  $17.9 \pm 2.6 \text{ l}\cdot\text{min}^{-1}$ ). These studies indicate that cardiac demand induced by sprint exercise may be as

high as one derived from maximal exercise eliciting  $\dot{V}O_{2\max}$  and that stroke volume remains elevated during a recovery phase. Nevertheless, when a repeated sprint protocol (5 x 30-s cycling sprints interspersed with 1 min of recovery) was followed by a 10-min recovery period, SV gradually decreased with passive recovery, reaching a similar value within 3 minutes compared to one obtained in a resting condition, whereas SV elevated throughout with active recovery (pedalling at 40W) (Crisafulli et al. 2004). This indicates that when recovery duration between high-intensity bouts is relatively long ( $\geq 3$  min), active recovery would result in a greater cardiac demand compared to passive recovery.

Despite the findings showing that recovery mode has an impact on acute cardiorespiratory/cardiovascular responses (Bogdanis et al. 1996b; Takahashi and Miyamoto 1998; Crisafulli et al. 2004) as well as repeated sprint performance as discussed in the previous section (Table 1.1), few studies have investigated effects of recovery mode on chronic adaptations using HIT. Ben Abderrahman et al. (2013) recently examined effects of recovery mode on changes in maximal aerobic velocity (MAV), time to exhaustion (TTE) and  $\dot{V}O_{2\max}$  using 7-week HIT programme (21 sessions in total) consisting of repeated 30-s runs at 100 to 110% MAV alternated with 30 sec of recovery. Whilst one group exercised at 50% MAV during the 30-s recovery periods (active recovery group), the other group rested passively (passive recovery group). Although the gains in performance (MAV and TTE) were observed irrespective of recovery mode, only the active recovery group increased  $\dot{V}O_{2\max}$  following the 7-week training. The discrepancy in training adaptations between the groups may be attributed to the difference in time spent at a high percentage of  $\dot{V}O_{2\max}$  relative to total exercise time during the training ( $> 90\%$   $\dot{V}O_{2\max}$ , AR vs. PR: 50.5 to 52.5% vs. 13.3 to 13.4%,  $P > 0.05$ ;  $> 95\%$   $\dot{V}O_{2\max}$ , AR vs. PR: 29.5 to 40.9% vs. 6.1 to 6.2%,  $P < 0.05$ ) (Ben Abderrahman et al. 2013). It has been suggested that time spent at a high percentage of

$\dot{V}O_{2\max}$  (e.g. > 90 % of  $\dot{V}O_{2\max}$ ) during exercise training is an important factor for improving maximal aerobic capacity (Billat 2001a; Thevenet et al. 2007; Zafeiridis et al. 2010), and therefore the greater aerobic demand induced by the active recovery likely resulted in the increased  $\dot{V}O_{2\max}$  in the study by Ben Abderrahman et al. (2013). Maximal oxygen uptake is most commonly measured to assess cardiorespiratory fitness of an individual (Bassett and Howley 2000), which is largely determined by oxygen supply (cardiac output) and its consumption and utilisation by skeletal muscle (Bassett and Howley 2000; Midgley et al. 2006a). Although endurance performance can be improved without the changes in  $\dot{V}O_{2\max}$  or  $\dot{V}O_{2\text{peak}}$  (Burgomaster et al. 2005, 2006) and thus improvements in  $\dot{V}O_{2\max}$  cannot alone explain those in endurance performance (Sloth et al. 2013), it is still an important determinant of endurance events (Midgley et al. 2006a) as the maximal oxygen uptake sets the upper limit for the rate of oxidative metabolism (Spriet 2006). To date, no studies have investigated effects of recovery mode on chronic adaptations to sprint-type (all-out) interval exercise training and thus it remains unknown whether active recovery is also favourable to aerobic adaptations in such training modality.

### **1.8 Effects of low-volume high-intensity interval training on physiological and performance adaptations**

Wingate-based high-intensity training (HIT) consisting of 4 to 6 x 30-s all-out maximal effort sprints interspersed with 4 min of recovery has been shown to be an effective training paradigm, inducing skeletal muscle and performance adaptations in as few as 6 sessions (Gibala and McGee 2008). It has been consistently found that 6 sessions of Wingate-based HIT over 2 weeks is sufficient to promote an increase in skeletal muscle oxidative capacity, glycolytic enzyme activity, buffering capacity and glycogen stores (Burgomaster et al. 2005,

2006; Gibala et al. 2006). In contrast, changes in  $\dot{V}O_{2\max}/\dot{V}O_{2\text{peak}}$  have been shown to be unaltered following 6 sessions of HIT (Burgomaster 2005, 2006), whilst others have found a 6-9% increase in  $\dot{V}O_{2\max}/\dot{V}O_{2\text{peak}}$  utilising the same HIT protocol (Bailey et al. 2009; Hazell et al. 2010; Astorino et al. 2012). However, when the number of sprint sessions is increased (12-18 sessions performed over 4-6 weeks) there is a consistent increase in  $\dot{V}O_{2\max}/\dot{V}O_{2\text{peak}}$  reported (Burgomaster et al. 2008; Trilk et al. 2011; Macpherson et al. 2011; Zelt et al. 2014; Table 1.2). Performance benefits of HIT are routinely seen after 6 sessions, with improvements in time to exhaustion, power production and cycling time trial reported (Burgomaster 2005, 2006; Gibala et al. 2006; Hazell et al. 2010; Astorino et al. 2012; Table 1.2). These improvements in performance have been related to the improvements in skeletal muscle oxidative capacity, buffering capacity and glycogen content (Burgomaster et al. 2005, 2006; Gibala et al. 2006). Furthermore the improvements in performance following 6 to 18 sessions of HIT have been shown to be similar to those resulting from traditional endurance training (60 to 120 min of continuous cycling at 65%  $\dot{V}O_{2\text{peak}}$ ) despite its markedly low-training volume (i.e. 2 to 3 min of all-out efforts per session) (Gibala et al. 2006; Burgomaster et al. 2008; Zelt et al. 2014). Together, the findings reported by recent Wingate-based studies suggest that HIT could be a time-efficient strategy to induce cardiorespiratory as well as skeletal muscle adaptations in a small timeframe. Although the underlying mechanisms responsible for training benefits derived from Wingate-based HIT have yet to be comprehensively revealed, a marked alteration in intramuscular homeostasis (e.g. phosphorylation potential) might play a key role in rapid training adaptations to the training (Gibala et al. 2009, 2012).

**Table 1.2** Summary of findings from recent Wingate-based studies

Study	N	Baseline $\dot{V}O_{2\max}$ (ml·min <sup>-1</sup> ·kg <sup>-1</sup> )	Study duration (wk)	$\dot{V}O_{2\max}$ changes	Performance changes
MacDougall et al. (1998)	12 M	51.0 ± 1.8	7	↑	PP & TW↑
Burgomaster et al. (2005)	6 M, 2F	44.6 ± 3.2	2	→	PP & TTE ↑, AP →
Burgomaster et al. (2006)	8 M	~48.7	2	→	PP, AP & TT↑
Gibala et al. (2006)	8 M	~52.6	2	NR	TT↑
Burgomaster et al. (2007)	8 M	50 ± 2	6	NR	TT↑
Burgomaster et al. (2008)	5 M, 5F	41 ± 2	6	↑	PP↑, AP↑
Babraj et al. (2009)	16M	50 ± 9	2	NR	TT↑
Bailey et al. (2009)	5M, 3F	42 ± 6	2	↑	TTE↑
Whyte et al. (2010)	10M	32.8 ± 1.4	2	↑	PP→ AP↑
Hazell et al. (2010)	35M, 13F	47 ± 6.7	2	↑	PP↑, AP↑, TT↑
Trilk et al. (2011)	14F	21.6 ± 1.1	4	↑	TW→
Macpherson et al. (2011)	6M, 4F	46.8 ± 5.1	6	↑	TT↑
Astorino et al. (2012)	11M, 9F	~43.4	2	↑	PP, AP & MP↑
Zelt et al. (2014)	11M	~48.6	4	↑	PP, AP & CP↑

N, number of participants; M, Male; F, Female;  $\dot{V}O_{2\max}$ , Maximal oxygen consumption; PP, AP, MP & TW,

peak power, average power, minimum power and total work, respectively during a single or repeated 30-s

Wingate tests; TTE, time to exhaustion; TT, time trial; CP, critical power; NR, not reported; ↑, Increase; →, No

change. Sample size only includes participants from 30-s HIT group except the study by Hazell et al. (2010).

Since Hazell et al. (2010) did not specify sample size or sex distribution for each group, the table includes

participants from all groups. If a study only reports absolute  $\dot{V}O_{2\max}$ , a relative value was estimated via

participants' total body mass.

## **1. 9 Molecular adaptations to high-intensity interval training**

It has been suggested that peroxisome proliferator-activated receptor gamma coactivator 1 $\alpha$  (PGC-1 $\alpha$ ) is regarded as a master regulator for mitochondrial biogenesis (Adhihetty et al. 2003; Laursen 2010; Gibala et al. 2012). A single session of Wingate-based HIT consisting of four 30-s sprints interspersed with 4-min recovery has been shown to increase PGC-1 $\alpha$  mRNA expression at the whole muscle level (Gibala et al. 2009) and nuclear PGC-1 $\alpha$  protein content (Little et al. 2011) in vastus lateralis 3 hours following the exercise. The magnitude of increase in the nuclear abundance of PGC-1 $\alpha$  (~66%) observed by Little et al. (2011) is comparable to that seen following endurance exercise consisting of 90 min cycling at 65%  $\dot{V}O_{2peak}$  (~54%, Little et al. 2010), suggesting similar acute molecular adaptations between the two exercise modalities. In terms of training adaptations, Gibala et al. (2006) has reported similar increases in the maximal activity of cytochrome c oxidase (COX) and COX subunits II and IV protein content in skeletal muscle following 2 weeks of Wingate-based HIT or endurance exercise training consisting of 90 to 120 min of continuous cycling at 65%  $\dot{V}O_{2peak}$ . Furthermore, similar findings in changes in mitochondrial protein content and activity (e.g. citrate synthase, 3-hydroxyacyl CoA dehydrogenase, pyruvate dehydrogenase) were reported following 18 sessions of Wingate-based HIT or 30 sessions of endurance training (40 to 60 min of continuous cycling at 65%  $\dot{V}O_{2peak}$ ) (Burgomaster et al. 2008). It has been demonstrated that when total energy expenditure or exercise time is matched, regulation of PGC-1 $\alpha$  gene expression is dependent upon exercise intensity regardless of exercise mode (Egan et al. 2010; Nordsborg et al. 2010). Egan et al. (2010) found a greater PGC-1 $\alpha$  mRNA expression in vastus lateralis following 36 min of continuous cycling at 80%  $\dot{V}O_{2peak}$  compared to 70 min of continuous cycling at 40%  $\dot{V}O_{2peak}$ . Similarly, PGC-1 $\alpha$  gene expression was greater when intermittent exercise (4 x 4-min cycling interspersed with 3 min

of recovery) was performed at 85%  $\dot{V}O_{2\max}$  as opposed to 70%  $\dot{V}O_{2\max}$  (Nordsborg et al. 2010). The findings reported by Egan et al. (2010) and Nordsborg et al. (2010) may explain the similarities between Wingate-based HIT and endurance exercise training in mitochondrial adaptations despite the large difference in exercise volume (Gibala et al. 2006; Burgomaster et al. 2008).

Among various candidates, adenosine monophosphate-activated kinase (AMPK), p38 mitogen-activated protein kinase (p38 MAPK) and calcium/calmodulin-dependent protein kinase (CaMK) have been suggested to be exercise-induced signalling kinases to activate PGC-1 $\alpha$  (Adhihetty et al. 2003; Laursen 2010; Gibala et al. 2012). AMPK kinase activity has been shown to be sensitive to exercise intensity (Fujii et al. 2000; Chen et al. 2000; Egan et al. 2010) due to increased alterations in intramuscular phosphorylation potential (i.e. AMP/ATP ratio) with exercise intensity (Chen et al. 2000, 2003). Chen et al. (2000) demonstrated an increased ratio of free AMP to ATP after a single 30-s cycling sprint. Likewise, there was a marked decrease in ATP concentration (by ~ 40%) following four 30-s sprints (Gibala et al. 2009). Considering that intramuscular ATP content has been reported to be maintained in type I muscle fibre but decreased in type II fibre following a single or repeated 30-s sprints (Esbjornsson-Liljedahl et al. 1999, 2002), a high level of muscle fibre recruitment may play a key role in mitochondrial adaptations following sprint interval training (Gibala et al. 2009, 2012).

p38 MAPK has also been suggested to be sensitive to metabolic stress during exercise (Little et al. 2011), and Kang et al. (2009) demonstrated that the degree of increases in p38 MAPK and PGC-1 $\alpha$  protein contents in rat vastus lateralis muscle were dependent upon reactive oxygen species (ROS) production. Nevertheless, similar activation of p38 MAPK was observed following low (40%  $\dot{V}O_{2\text{peak}}$ ) - or high (80%  $\dot{V}O_{2\text{peak}}$ ) - intensity cycling (Egan et al.

2010) and therefore it has yet to be determined whether exercise intensity or metabolic stress plays a role in activating this exercise-responsive kinase.

Whilst isoform expression or phosphorylation of CaMK II has been shown to increase following sub-maximal-intensity exercise (Rose et al. 2007; Egan et al. 2010), phosphorylation of CaMK II was not significantly increased following Wingate-based HIT despite increases in phosphorylated AMPK and p38 MAPK, which were followed by an increased PGC-1 $\alpha$  gene expression (Gibala et al. 2009). The lack of the increase in CaMK II might be attributed to its short exercise-duration (i.e. 30 sec) since an increased Ca<sup>2+</sup> flux during prolonged muscle contraction has been suggested to activate calcium-sensitive protein kinases (Adhihetty et al. 2003; Kang et al. 2009; Laursen 2010). Rose et al. (2007) observed increases in maximal CaMK II activity and CaMK II kinase isoform expression after 3 weeks of endurance exercise training consisting of dynamic knee extension exercise for 1 to 2 h per session (Rose et al. 2007).

Taken together, it appears that the activation of PGC-1 $\alpha$  is mediated by different mechanisms with AMPK (and possibly p38 MAPK) being sensitive to exercise intensity and CaMK being activated by prolonged muscle contraction.

## **1.10 Training Adaptations: Central or Peripheral?**

### *1.10.1 High-intensity aerobic interval training*

Central adaptations to aerobic-type interval training have been consistently reported. Daussin et al. (2007, 2008) demonstrated that 8-week interval training consisting of 1-min cycling at 90% of  $\dot{V}O_{2\max}$  separated by 4-min active recovery at lactate threshold (3d $\cdot$ wk<sup>-1</sup>, 24 sessions in total) brought about increased maximal cardiac output and stroke volume as well as peripheral adaptations such as increases in maximal a- $\bar{v}$  O<sub>2</sub> difference, skeletal muscle



mitochondrial oxidative capacities and capillary density. Matsuo et al. (2014) have also shown that their 8-week interval training programme consisting of three 3-min cycling at 80-90%  $\dot{V}O_{2\max}$  alternated with 2-min cycling at 50%  $\dot{V}O_{2\max}$  increases stroke volume and left ventricular mass by 12.1 and 8.0%, respectively in addition to a 22.5% increase in  $\dot{V}O_{2\max}$ . Furthermore, Helgerud et al. (2007) showed that 8-week aerobic interval training performed at 90-95%  $HR_{\max}$  resulted in improvements in stroke volume by approximately 10%, while such benefits were not observed following continuous aerobic exercise at lower intensities (70-85%  $HR_{\max}$ ). In these studies, total work or duration of the high-intensity aerobic interval training was either identical (Daussin et al. 2007, 2008; Helgerud et al. 2007) or approximately 50% (Matsuo et al. 2014) compared with those of continuous endurance training at lower intensities, suggesting that exercise intensity rather than volume or duration is a key factor to improve cardiac function. Nevertheless, studies to date suggest that sub-maximal rather than maximal or supra-maximal intensity may be optimal to achieve cardiac benefits of exercise training.

### *1.10.2 Sprint interval training*

In contrast to aerobic-type interval training, there have been mixed findings in terms of central adaptations following maximal- or supra-maximal- intensity interval training. Macpherson et al. (2011) reported that training benefits derived from Wingate-based HIT was attributed to peripheral adaptations. Following 6 weeks of either Wingate-based HIT or endurance training (ET: 30 to 60 min of continuous exercise at 65%  $\dot{V}O_{2\max}$ ), they observed a similar magnitude of training gains in  $\dot{V}O_{2\max}$  (Wingate vs. ET: 11.5% vs. 12.5%) and 2000-m run time-trial performance (Wingate vs. ET: 4.6% vs. 5.9%). However, the underlying mechanisms responsible for these training benefits were different between the training groups. While only the ET increased maximal cardiac output (~ 9%), maximal arterial-mixed venous

O<sub>2</sub> difference was increased (7.1%) and decreased (7.1%) following the Wingate-based HIT and ET, respectively (Macpherson et al. 2011). In contrast, Trilk et al. (2011) saw increases in stroke volume as well as  $\dot{V}O_{2\max}$  but not in arterial-venous O<sub>2</sub> difference during sub-maximal exercise following 4 weeks of Wingate-based HIT. Furthermore, when exercise intensity is set relative to  $\dot{V}O_{2\max}$  as opposed to all-out sprinting (e.g. Wingate-based HIT), mixed findings have been also reported. Matsuo et al. (2014) showed increased stroke volume and left ventricular mass (by 6.5 and 5.3%, respectively) following 8 weeks of sprint interval training consisting of 7 x 30-s cycling at 120%  $\dot{V}O_{2\max}$  interspersed with 15-s rest periods. Similarly, Esfandiari et al. (2014) observed approximately 13% gains in cardiac output and stroke volume during sub-maximal exercise due to an increase in end-diastolic volume following 2 weeks of HIT consisting of 8 to 12 x 60-s cycling at 95 to 100%  $\dot{V}O_{2\max}$  separated by 75-s of active recovery at 10%  $\dot{V}O_{2\max}$ . On the other hand, whist Jacobs et al. (2013) employed the same training protocol as Esfandiari et al. (2014) over 2 weeks, they did not see a gain in maximal cardiac output and changes in  $\dot{V}O_{2\max}$  were associated with increases in skeletal muscle mitochondrial content and function (COX). Baseline fitness level might account for the changes in cardiac function with HIT. The participants in the study by Esfandiari et al. (2014) possessed lower baseline  $\dot{V}O_{2\max}$  ( $39.5 \pm 7.1 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) compared to those studied by Jacobs et al. (2013) ( $43 \pm 6 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ). Similarly, Trilk et al. (2012) recruited sedentary, overweight/obese women (baseline  $\dot{V}O_{2\max}$ :  $21.6 \pm 1.1 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ), whereas the participants studied by Macpherson et al. (2011) were recreationally active men and women ( $46.8 \pm 5.1 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ). Differences in methods could also explain the discrepancies given that while Esfandiari et al. (2014) and Trilk et al. (2011) assessed cardiac measures during steady-state sub-maximal exercise, Macpherson et al. (2011) and Jacobs et al. (2013) investigated maximal values (i.e. maximal cardiac output).

Several reasons seem to explain the lack of consistency in central adaptations following supra-maximal- or maximal- intensity interval training. Firstly, due to its intensity, supra-maximal interval training often results in short exercise and long recovery duration, respectively (e.g. sprint: rest ration of typical Wingate-based HIT is 30s: 240s or 1:8). Although cardiorespiratory demand could approach its maximal (e.g. a high percentage of  $\dot{V}O_{2\max}$  or  $HR_{\max}$ ) during sprints, its work: rest ratio does not seem to allow a sustained or constant cardiac demand. Whilst Buchheit et al. (2012) found that  $\dot{V}O_2$  and HR reached  $90.4 \pm 2.8\% \dot{V}O_{2\max}$  and  $96 \pm 3\% HR_{\max}$ , respectively during a sprint protocol consisting of 6 x 30- s all-out cycling sprints separated by 2 min of passive recovery (sprint: work ratio: 1:4) in 10 trained male cyclists ( $\dot{V}O_{2\max}$ :  $60.1 \pm 5.9 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ), session-average  $\dot{V}O_2$  and HR were  $48.0 \pm 4.1\% \dot{V}O_{2\max}$  and  $82 \pm 3\% HR_{\max}$ , respectively. Indeed, the time spent above 90%  $\dot{V}O_{2\max}$  was only  $22 \pm 21$  sec, indicating insufficient stimulus for the cardiorespiratory system (Buchheit et al. 2012). Although Ben Abderrahman et al. (2013) did not assess central adaptations, increases in  $\dot{V}O_{2\max}$  were associated with time spent above 90%  $\dot{V}O_{2\max}$  during HIT consisting of repeated 30-s runs at 100 to 110% MAV (Ben Abderrahman et al. 2013). Moreover, short recovery (15 sec) during the sprint interval training employed by Matsuo et al. (2014) resulted in greater peak and average HRs (179 and 161 bpm) compared with those of continuous aerobic exercise training consisting of 40 min of cycling at 60 to 65%  $\dot{V}O_{2\max}$  (160 and 152 bpm, respectively), which increased  $\dot{V}O_{2\max}$  but not LV mass or stroke volume. Therefore, it could be argued that when performing short high-intensity interval training ( $\leq 60$  sec), a greater cardiac demand would be ensured by reducing recovery duration (Matsuo et al. 2014) or increasing recovery intensity (Ben Abderrahman et al. 2013).

### **1.11 Effects of sprint duration on training gains**

Although Wingate-based HIT has been shown to produce comparable metabolic and physiological adaptations to those obtained from traditional aerobic exercise training despite its low training volume (2 to 3 min of all-out efforts per session), typical Wingate-based HIT may not be necessarily time-efficient if warm-up and recovery periods are included, amounting to ~ 30 minutes per session (Gibala et al. 2006; Burgomaster et al. 2008). This means that its total time commitment can be comparable to public exercise guidelines (30 min of moderate exercise most days of the week) recommended by major organisations (e.g. ACSM) (Haskell et al. 2007). In addition, considering the very intense nature of all-out sprinting, reducing the duration of sprint may make the training more practical for a wider range of populations (Gibala and McGee 2008). Parolin et al. (1999) found that while glycogenolysis was fully activated during the first 15 sec of a 30-s maximal sprint, it was strongly attenuated during the second 15 seconds. Likewise, the majority of PCr utilisation has been shown to complete within 10 sec during a maximal sprint (Bogdanis et al. 1996a; 1998). Furthermore, it has been demonstrated that the contribution from aerobic metabolism to sprint performance increases with successive bouts irrespective of sprint duration (6 to 30 sec) when sprints are separated by 30-s to 4-min recovery (Gaitanos et al. 1993; Bogdanis et al. 1996a; Bogdanis et al. 1998; Parolin et al. 1999; Hargreaves et al. 1998). Together, it could be assumed that a shorter sprint protocol ( $\leq 15$  sec) would result in a similar metabolic demand compared to that seen in typical Wingate-based HIT and reducing sprint duration may not diminish overall training gains. Indeed, Hazell et al. (2010) demonstrated that a reduced-exertion sprint interval training consisting of 4 to 6 10-s cycling sprints separated by 2- or 4-min recovery was as effective as typical Wingate-based HIT in improving  $\dot{V}O_{2\max}$ , 5-km time trial and single 30-s Wingate performance. Similarly, Zelt et al. (2014) have recently

demonstrate that 4 to 6 15-s sprints interspersed with 4.75 min of recovery improve aerobic capacities such as  $\dot{V}O_{2\text{peak}}$ , critical power and lactate threshold to a similar extent compared to 30-s Wingate-based HIT. Hazell et al. (2010) saw a greater training intensity (as reflected by improved reproducibility of power) in their short sprint interval training groups compared to the 30-s Wingate-based HIT group, and suggested that the level of power production rather than sprint duration may play a role in training adaptations to sprint interval training. Nevertheless, since neither training volume (sprint duration) nor sprint-to-rest ratio was matched between the groups in the study by Hazell et al. (2010) or Zelt et al. (2014), it remains unknown whether the improved power reproducibility seen in the reduced-volume sprint interval training (Hazell et al. 2010) is due to shorter sprint duration, greater sprint-to-rest ratio or both. Therefore, further studies are required to determine whether reduced-volume of sprint interval training with the same sprint: rest ratio as the conventional 30-s Wingate-based HIT (i.e. 1:8) also ensures better power maintenance during training and results in comparable training benefits.

## **1.12 Time course of physiological and performance adaptations to high-intensity interval training**

### *1.12.1 Time course of changes in cardiorespiratory function*

Whilst an improved  $\dot{V}O_{2\text{max}}/\dot{V}O_{2\text{peak}}$  has been robustly seen when Wingate-based HIT are performed more than 4 weeks (MacDougall et al. 1998; Burgomaster et al. 2008; Macpherson et al. 2011; Trilk et al. 2011), this may be attributed to initial training adaptations.

Burgomaster et al. (2008) observed a 7.3% increase in  $\dot{V}O_{2\text{peak}}$  following 3 weeks of Wingate-based HIT, whereas no further improvement was confirmed with additional 3 weeks of the training. Furthermore, Astorino et al. (2013) examined magnitude and time course of changes in maximal oxygen uptake in response to low- or high-intensity aerobic interval training over

12 weeks. In their study, interval training was consisted of six to ten 60-s cycling bouts separated by 60 to 75 sec of recovery where one group performed at 80 to 90%  $\dot{V}O_{2\max}$  (high-intensity group, HI), whereas the other group performed at 60 to 80%  $\dot{V}O_{2\max}$  (low-intensity group, LO). Following the 12-week training interventions, the magnitude of increase in  $\dot{V}O_{2\max}$  was not different between the groups (HI vs. LO:  $21.9 \pm 11.9$  vs.  $22.3 \pm 9\%$ ), however, greater gains in  $\dot{V}O_{2\max}$  occurred in HI group during the initial 3 weeks compared to LO group (60% vs. 20% of the overall gain), suggesting that the improvements of cardiorespiratory function were rapidly induced by the high-intensity training (Astorino et al. 2013). Using Sprague-Dawley rats, Wisløff et al. (2001) employed high-intensity aerobic interval training consisting of repeated 8-min runs at 85-90%  $\dot{V}O_{2\max}$  alternated with 2 min of active recovery at 50-60%  $\dot{V}O_{2\max}$  over 13 weeks, and demonstrated that the majority of the increase in  $\dot{V}O_{2\max}$  occurred within 4 weeks. Considering that the training rats increased their  $\dot{V}O_{2\max}$  in parallel with improvements in myocardial contractility and  $Ca^{2+}$  - handling, the changes of  $\dot{V}O_{2\max}$  in their study likely reflects those of cardiac function (Wisløff et al. 2001). Likewise, Kemi et al. (2004) employed the same high-intensity training protocol as Wisløff et al. (2001) and showed close relationships between improvements in  $\dot{V}O_{2\max}$  and those in myocardial variables (e.g.  $\dot{V}O_{2\max}$  vs. cardiomyocyte length, shortening and relaxation:  $r = 0.92, 0.88$  and  $-0.92$ , respectively), and both  $\dot{V}O_{2\max}$  and myocardial adaptations levelled off within 6 to 8 weeks. This is in line with the study by Astorino et al. (2013) who observed no difference in  $\dot{V}O_{2\max}$  between week 6 and 12 weeks in the high-intensity training group. Although the increase in  $\dot{V}O_{2\max}$  is not exclusively explained by central adaptations and it also reflects peripheral adaptations (Daussin et al. 2008; Macpherson et al. 2011, Jacobs et al. 2013), it seems that the time course of changes in  $\dot{V}O_{2\max}$  in response to HIT at least partially reflects that of cardiac adaptations. Contrary to the study by Astorino et al. (2013), Kemi et al. (2005) demonstrated that improvements in  $\dot{V}O_{2\max}$  and cardiomyocyte contractility are

intensity-dependent. Following 10 weeks of high-intensity training (5 x 8-min uphill runs at 85 to 90%  $\dot{V}O_{2\max}$ ),  $\dot{V}O_{2\max}$  was increased to a greater extent than moderate-intensity training (the identical training protocol but performed at 65 to 70%  $\dot{V}O_{2\max}$ ) (high vs. moderate: 71 vs. 28%), mainly owing to greater left ventricular hypertrophy (14 vs. 5%) and cardiomyocyte contractility (fractional shortening) (45 vs. 23%). Since Kemi et al. (2005) studied rats, their findings are not able to be directly compared with those obtained from Astorino et al. (2013), intensity-dependent training adaptations observed by Kemi et al. (2005) might be explained by their greater training volume (40 min of high-intensity running per session, 5 days per week) compared to the study by Astorino et al. (2013) (6 to 10 min of high-intensity cycling per session, 3 days per week). Moreover, Kemi et al. (2005) also showed that despite the differences in cardiorespiratory and myocardial adaptations to the high- vs. moderate-intensity training, improvements in endothelial-mediated vasodilation were not intensity-dependent. Therefore, it could be also assumed that in the study by Astorino et al. (2013), the HI and LO training groups might have improved endothelial-dependent vasodilation to a similar extent, allowing a similar improvement in arterial compliance to increase oxygen delivery to skeletal muscle (Kemi et al. 2004, 2005), whereas the time course of cardiac adaptations was intensity-dependent. Although further long-term studies are clearly required, progressive increases in relative exercise load (volume or intensity, or both) may be necessary to see continuous improvements in cardiac function and thus  $\dot{V}O_{2\max}$  when performing HIT, provided that individuals have not reached their maximal potential (Wisloff et al. 2009).

#### *1.12.2 Time course of changes in peripheral function*

Burgomaster et al. (2007) demonstrated divergent effects of Wingate-based HIT on metabolic and performance adaptations using 6 weeks of Wingate-based HIT. While increases in the

protein contents of cytochrome *c* oxidase subunit 4 (COX4), glucose transporter 4 (GLUT 4) and monocarboxylate transporter 4 (MCT 4) were observed within 1 week, the contents of these proteins remained unchanged thereafter. Conversely, effects of Wingate-based HIT on MCT 1 protein content and time-trial performance were only observed after 6 weeks, and fatty acid transport proteins such as fatty acid translocase (FAT/CD 36) and plasma membrane-associated fatty acid binding protein (FABPpm) were unaltered throughout 6 weeks (Burgomaster et al. 2007). Considering that Burgomaster et al. (2007) indicated that the increase in skeletal muscle oxidative capacity, as reflected by the increase in COX 4 protein content, plateaued after 1 week and the gain in  $\dot{V}O_{2\max}$  induced by Wingate-based HIT has been associated with peripheral factors (arterial-venous  $O_2$  difference) (Macpherson et al. 2011), the plateau in  $\dot{V}O_{2\text{peak}}$  improvement seen after 3 weeks in the study by Burgomaster et al. (2008) might reflect a levelling-off of skeletal muscle oxidative adaptations. Nevertheless, Trilk et al. (2011) has shown that 4 weeks of Wingate-based HIT improves stroke volume although this might be attributed to their overweight/obese participants with low baseline  $\dot{V}O_{2\max}$  ( $21.6 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ). Furthermore, improvements in mitochondrial function (e.g. CS, PDH) did not accompany changes in  $\dot{V}O_{2\text{peak}}$  following 2 weeks of Wingate-based HIT (Burgomaster et al. 2005, 2006), indicating that the improvements in cardiorespiratory function derived from sprint interval training cannot be totally attributed to peripheral factors (Sloth et al. 2013).

To date, physiological and performance adaptations to Wingate-based HIT have been measured at only two different time points over a relatively short period (6 weeks) (Burgomaster et al. 2007, 2008), and thus further research is required to determine the time course of training gains over a longer timeframe.



### **1.13 Aims**

The major aims of this PhD are to determine 1) the effects of recovery intensities on cardiorespiratory responses and repeated sprint performance during Wingate-based HIT, 2) the effects of recovery mode on physiological and performance adaptations to Wingate-based HIT over 2 weeks and 3) the effects of sprint duration and time course of physiological and performance adaptations to HIT over 9 weeks

Despite well-established training benefits derived from Wingate-based HIT (e.g. Gibala et al. 2006; Burgomaster et al. 2008), little attention has been paid to recovery periods during the training modality. Although previous studies have indicated that active recovery facilitates power maintenance during repeated 30-s Wingate tests (Bogdanis et al. 1996b; Spierer et al. 2004; Lopez et al. 2014), these studies merely compared active vs. passive but not the intensity of recovery, and only Bogdanis et al. (1996b) investigated the effects of recovery mode on cardiorespiratory responses during two 30-s Wingate tests separated by 4 min of recovery. Therefore, it remains unknown whether the intensity of recovery has an impact on overall cardiorespiratory responses and performance during typical Wingate-based HIT (4 to 6 x 30-s sprints interspersed with 4 min of recovery against 7.5% bodyweight). Furthermore, no previous studies have examined effects of recovery mode on physiological and performance adaptations to sprint-type (all-out) interval training such as Wingate-based HIT. What is more, although reduced-volume of sprint interval training (10 to 15 sec of repeated sprints) has been shown to be as effective as typical Wingate-based HIT in improving physiological and performance parameters (Hazell et al. 2010; Zelt et al. 2014), sprint-to-rest ratio between the reduced-volume sprint training and Wingate-based HIT was not matched in these studies. Thus, it has yet to be determined whether sprint duration itself or sprint-to-rest ratio is a driving factor in the training adaptations. Finally, considering that the majority of

the previous Wingate-based studies have been conducted over 2 to 6 weeks, further research is required to investigate the time course of physiological and performance adaptations to Wingate-based HIT beyond that period (i.e. > 6 weeks).

In short, this PhD would contribute to the advances of knowledge in sport and exercise physiology regarding how the arrangements of recovery modality and sprint duration affect physiological and performance adaptations to sprint interval training as well as the time course of those adaptations.

The main hypotheses are that –

- Cardiorespiratory responses would be increased with the increase in recovery intensity during typical Wingate-based HIT, whereas active recovery would improve repeated sprint performance regardless of the intensity compared with passive recovery
- Greater endurance performance adaptations would be induced by active recovery due to a higher aerobic metabolic demand during the training compared with passive recovery.
- When sprint-to-rest ratio is matched, the duration of sprint would not have a major impact on overall training adaptations.
- The majority of adaptations to sprint interval training would be attributed to those achieved during the initial phases.

# **Chapter 2**

## **General Methodology**

## **2.1 Participants**

Healthy active adults aged 18 to 35 years who took part in a minimum of 3-h exercise per week were recruited via email or verbal communication in all studies. Seven male participants completed study 1, whereas 14 (M: 9; F: 5) and 25 (M: 18; F: 7) male and female participants completed study 2 and 3, respectively. All participants completed a Physical Activity Readiness Questionnaire (PARQ) to ensure that there were no underlying health issues, and they were fully informed both verbally and in writing about the study before giving their informed consent. All were physically active who met public exercise guidelines (i.e. a minimum of 150 min of moderate exercise per week) recommended by major organisations (e.g. ACSM) (Haskell et al. 2007), but none of them were participating in regular sporting competitions during the period of any study. All studies were approved by the Institutional Ethics Committee and were carried out in line with the Declaration of Helsinki.

## **2.2 Experimental procedures**

### **2.2.1 Body composition**

The body mass, fat percentage and stature of each participant were recorded to the nearest 0.1kg, 0.1% and 0.1cm, respectively. Body mass and fat percentage were measured on a calibrated bioelectrical impedance meter (SC-330ST Tanita Body Composition Analyser, Tanita Europe BV, Amsterdam, Netherlands), whereas stature was measured using a stadiometer (SECA 216 Mechanical Stadiometer, Hamburg, Germany). Bioelectrical impedance analysis (BIA) has been shown to be a reliable method of measuring body composition with < 1% error on repeated measurements (Dehghan and Merchant 2008; von Hurst et al. 2015). In order to minimise the effect of food and fluid intake or exercise on body composition measurement on the bioelectrical impedance meter (Dehghan and Merchant

2008), all participants reported to the laboratory after a 4-h fast and they were asked to refrain from any form of intense exercise for 24 h prior to the analysis of body composition. When this has been done, BIA has been shown to be as reliable as dual-energy X-ray absorptiometry (DEXA) scanning for analysis of fat composition (Beeson et al. 2010). Nevertheless, it has been also demonstrated that BIA underestimates body fat percentage by 2% when compared with air displacement plethysmography (ADP) and DEXA in a wide range of ages (19-71 years), BMI (19-38 kg/m<sup>2</sup>) and body fat percentage ( $\leq 12\%$  to  $>40\%$ ) (von Hurst et al. 2015). Therefore, one should bear in mind that although BIA would provide a reliable value in a controlled condition, it may underestimate body fat percentage compared to other measurements.

### 2.2.2 Blood pressure

Blood pressure measurements were performed in an attempt to determine effects of training intervention on cardiovascular function. Both systolic and diastolic blood pressure have been shown to be related to peripheral vascular resistance until age 50 years (Franklin et al. 1997). Blood pressure can be also categorised into pulsatile and steady components with the former being estimated by pulse pressure (PP) and the latter being estimated by mean arterial pressure (MAP) (Franklin et al. 1997; Sesso et al. 2000). PP represents the variation in blood pressure and is mainly dependent upon large artery stiffness (Benetos et al. 1997; Franklin et al. 1997), whereas MAP represents the steady flow of blood through the aorta and its arteries and is determined by cardiac output and vascular resistance (Franklin et al. 1997; Sesso et al. 2000). Sprint interval training has been shown to improve peripheral artery (popliteal artery) distensibility and flow-mediated dilation in young healthy male and female adults in the absence of changes in central artery (carotid artery) stiffness (Rankobowchuk et al. 2008). Therefore, changes in blood pressure variables with high-intensity training may reflect

improvements in peripheral vascular structure and function when young healthy adults are studied. Prior to obtaining a blood pressure reading, all participants were required to rest in a sitting position for five minutes to allow the normalisation of blood pressure. Blood pressure was then obtained from their left arm on a flat table surface using an automatic blood pressure monitor (Watch BP<sup>®</sup> office, Microlife Health Management Ltd., Cambridge, UK). The blood pressure was measured three consecutive times interspersed with a minimum of 15 sec and the average of the three measurements was recorded. Prior to each blood pressure measurement, all participants were asked to refrain from any form of intense physical activity for 24 h to minimise an acute effect of exercise on blood pressure (Cardoso et al. 2010).

### 2.2.3 Determination of peak oxygen uptake and other performance variables during incremental tests

Peak oxygen uptake ( $\dot{V}O_{2peak}$ ) is defined as the highest rate at which oxygen can be taken up and utilised by the body during maximal exercise (Bassett and Howley 2000). This parameter is most commonly used to assess the cardiorespiratory fitness of an individual in the field of exercise physiology (Bassett and Howley 2000; Legaz Arrese et al. 2005). In study 1, the determination of  $\dot{V}O_{2peak}$  was made as aerobic capacity has been shown to influence repeated sprint performance (Hamilton et al. 1991; Tomlin and Wenger 2002; Bishop and Edge 2006), whereas  $\dot{V}O_{2peak}$  was determined to examine the effect of training intervention on cardiorespiratory function in study 2 and 3.

#### 2.2.3.1 Study 1

Participants performed an exhaustive incremental cycling test on a cycle ergometer (Monark Ergomic 874E, Varberg, Sweden) to determine  $\dot{V}O_{2peak}$  via breath by breath analysis (Metalyzer<sup>®</sup>3B gas analyser, Cortex, Leipzig, Germany). The test commenced at an initial

power output of 60W, with an additional 30W increase every minute until the participants could not maintain a speed of 60 rpm or until volitional exhaustion occurred despite strong verbal encouragement. After the incremental test, an additional supra-maximal verification test was performed to ensure that true  $\dot{V}O_{2peak}$  had been elicited. Participants rested for 5 minutes either passively or actively (unloaded cycling). They then cycled again until they reached the limit of tolerance (~2min) at a work rate equivalent to one stage higher (30W higher) than that of the last stage in the incremental test (Rossiter et al. 2006). A high correlation between peak  $\dot{V}O_2$  achieved during the incremental and verification tests was obtained using a linear regression model ( $r \geq 0.99$  in both relative and absolute values), and the difference in peak  $\dot{V}O_2$  between the two tests ( $77.4 \pm 36.4 \text{ ml} \cdot \text{min}^{-1}$  or  $1.0 \pm 0.5 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) was less than the conventional concept of a plateau in  $\dot{V}O_2$  (i.e.  $\leq 150 \text{ ml} \cdot \text{min}^{-1}$  or  $\leq 2.1 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ; Duncan et al. 1997) suggesting the attainment of a true  $\dot{V}O_{2peak}$ .

Respiratory gas exchange measures were averaged every 10s with  $\dot{V}O_{2peak}$  calculated as the highest oxygen consumed over a 10-s period, while power output elicited at  $\dot{V}O_{2peak}$  was defined as the maximal power output ( $P_{max}$ ). Heart rate was recorded throughout using a heart rate monitor (Polar Electro, Kempele, Finland) and was averaged every 5s. Maximal heart rate ( $HR_{max}$ ) was defined as the highest heart rate recorded over a 5-s period. Recovery intensities (i.e. 20, 30 and 40% of  $\dot{V}O_{2peak}$ ) employed during repeated 30-s Wingate tests were determined according to the linear relationship between each individual's  $\dot{V}O_2$  and work rate during the incremental test.

### 2.2.3.2 Study 2 and 3

In study 2 and 3, participants performed an exhaustive incremental cycling test on a cycle ergometer (Monark Ergomedic 874E, Varberg, Sweden) to determine  $\dot{V}O_{2peak}$  via breath by breath analysis (Metalyzer<sup>®</sup>3B gas analyser, Cortex, Leipzig, Germany) before and after 2

weeks (study 2) or 9 weeks (study 3) of HIT. The test commenced at an initial power output of 70W, with an additional 35W increase every 3 minutes until volitional exhaustion or the participants could not maintain 70 rpm despite strong verbal encouragement. Exercise duration at exhaustion was recorded to the nearest second and defined as time to exhaustion (TTE), and maximal power output ( $P_{max}$ ) was calculated from the last completed work rate, plus the fraction of time spent in the final non-completed work rate multiplied by the work rate increment (i.e. 35W) (Achten and Jeukendrup 2003). Respiratory gas exchange measures were averaged every 30s with  $\dot{V}O_{2peak}$  calculated as the highest oxygen consumed over a 30-s period. Heart rate was recorded throughout using a heart rate monitor (Polar Electro, Kempele, Finland) with maximal heart rate (HR max) defined as the highest heart rate recorded over a 30s period. A slower increment rate (35 watts per 3 min) was employed in these studies to assess changes in substrate utilisation with the training (see the section 2.2.8 “Crossover point”).

## 2.2.4 Assessment of repeated sprint performance

### 2.2.4.1 Equipment

All participants performed cycle sprints on a Monark cycle ergometer (Monark Ergonomic 894E, Varberg, Sweden) and power output (peak, average and minimum power) was calculated automatically using Monark software (Monark Anaerobic Test Software version 2.24.2, Monark Exercise AB). Total work was determined by integrating power output recorded every second, and power drop rate across sprints was determined using the formulas shown in the sections below.



#### 2.2.4.2 Study 1

Participants performed 4 x 30-s cycle sprints against 7.5% bodyweight interspersed with 4 minutes of recovery on 4 different days separated by at least 48 h. Upon cessation the workload was adjusted to a given recovery intensity (20, 30, or 40%  $\dot{V}O_{2peak}$ ), which had been randomly allocated, and the participants cycled at this intensity for 230 sec. 10 sec before the next sprint, the workload was adjusted again to 7.5% of the participants' bodyweight. In the case of passive recovery, the participants remained still on the bike for 240 sec. The recovery intensities employed in this study are the most commonly reported during repeated cycle sprints (Bogdanis et al. 1996b; Dupont et al. 2004, 2007; Dorado et al. 2004; Spierer et al. 2004; Spencer et al. 2006; 2008); however, it is unknown whether the intensity of recovery affects overall performance during repeated 30-s Wingate tests since the previous studies merely compared active or passive but not intensity of recovery (Bogdanis et al. 1996b; Spierer et al. 2004; Lopez et al. 2014). Sprint performance was assessed via peak and average power produced during each of the four 30-s sprints. Power drop rate of peak or average power across the 4 sprints was also determined using the following formulas;

Peak power drop:  $(PP^{S2, S3 \text{ or } S4} - PP^{S1}) / PP^{S1} \times 100$ , where  $PP^{S1}$  is peak power of sprint 1 and  $PP^{S2, S3 \text{ or } S4}$  is peak power of sprint 2, 3, or 4.

Average power drop:  $(AP^{S2, S3 \text{ or } S4} - AP^{S1}) / AP^{S1} \times 100$ , where  $AP^{S1}$  is average power of sprint 1 and  $AP^{S2, S3 \text{ or } S4}$  is average power of sprint 2, 3, or 4.

#### 2.2.4.3 Study 2

All participants performed 6 sessions of 4 to 6 x 30-s cycle sprints against 7.5% bodyweight, interspersed with 4 min of recovery over 2 weeks. However, while one group cycled at an intensity equivalent to 40% of  $\dot{V}O_{2peak}$  (active recovery group, ARG) during the 4-min recovery, the other remained still on the bike (passive recovery group, PRG). The recovery

intensity for ARG was derived from the linear relationship between each individual's  $\dot{V}O_2$  and work rate during the incremental test. The participants in PRG were allowed to cycle unloaded at a low speed if they wanted to. In study 1, this recovery intensity has been shown to facilitate better maintenance of power production compared to passive recovery or lower recovery intensity (20%  $\dot{V}O_{2peak}$ ) with successive sprints while increasing cardiorespiratory demand. Therefore, it was assumed that the recovery intensity equivalent to 40% $\dot{V}O_{2peak}$  would promote greater training adaptations compared with passive recovery.

Average values of peak and average power over the first 4 sprints were calculated in the first and last training sessions in order to examine effects of respective training on repeated sprint performance as well as difference between the training groups (i.e. ARG vs. PRG).

#### 2.2.4.4 Study 3

Participants were assigned to either 15-s training group (15TG) or 30-s training group (30TG). Both groups performed the same number of cycle sprints over 9 weeks; however, sprint and recovery duration were different between the groups. While 15TG performed 4 to 6 x 15-s cycle sprints interspersed with 2-min recovery, the duration of sprint and recovery in 30TG were 30 sec and 4 min, respectively (i.e. the same sprint: rest ratio). Both groups performed active recovery (40% $\dot{V}O_{2peak}$ ) during their respective recovery periods. The recovery intensity was derived from the linear relationship between each individual's  $\dot{V}O_2$  and work rate during the incremental test, and it was recalculated for each participant every 3 weeks according to  $\dot{V}O_{2peak}$  measurement. In this study, total work across the first 4 sprints was calculated to determine the difference in training volume between the groups (i.e. 15-s vs. 30-s sprints) in addition to peak power over the 4 sprints. Furthermore, to assess the reproducibility of power during the training, power drop rate across the 4 sprints was also calculated using the following formula;

Reproducibility of power over the 4 sprints:  $((PO_1 + PO_2 + PO_3 + PO_4) / 4) / \text{best PO} \times 100$ , where PO is power output (either peak or average) (Hazell et al. 2010).

Repeated sprint performance was assessed every 6 sessions over 9 weeks (4 times in total).

## 2.2.5 Assessment of cardiorespiratory responses during repeated sprints

### 2.2.5.1 Equipment

In study 1 and 2, cardiorespiratory variables were recorded via the breath by breath oxygen analyser (Metalyzer®3B gas analyser, Cortex, Leipzig, Germany) and portable heart rate monitor (Polar Electro, Kempele, Finland), whereas Bioharness (Bioharness™ 2, Zephyr Technology, MD, USA) was used to monitor HR in study 3. Where the use of the oxygen analyser was required, the oxygen (O<sub>2</sub>) and carbon dioxide (CO<sub>2</sub>) analyser systems were calibrated using ambient air with a gas mixture of known O<sub>2</sub> and CO<sub>2</sub> immediately before each test. Partial O<sub>2</sub> and CO<sub>2</sub> in ambient air were assumed to be 20.93% and 0.03%, respectively. The reference gas concentrations in O<sub>2</sub> and CO<sub>2</sub> used for the calibration were 17.10% and 5.00%, respectively. The turbine flowmeter of the Metalyzer®3B gas analyser was calibrated using a 3-L calibration syringe (Hans Rudolph, inc., Kansas city, USA).

### 2.2.5.2 Study 1

The main purpose of this study was to determine oxygen demand during sprints and recovery periods across the four different recovery conditions (passive, 20, 30 and 40%  $\dot{V}O_{2\text{peak}}$ ). Oxygen uptake and HR were averaged every 5 seconds during the sprint protocol, and average values were expressed as percentage of  $\dot{V}O_{2\text{peak}}$  and HR<sub>max</sub> determined in the incremental test, respectively. During recovery, each  $\dot{V}O_2$  or HR was divided by  $\dot{V}O_{2\text{peak}}$  or HR<sub>max</sub>, and the slope of  $\dot{V}O_2$  or HR versus time was determined for each recovery condition using a second-order polynomial regression (Figure 3.3A&B; Cohen-Solal et al. 1995).

Although the decrease in  $\dot{V}O_2$  was well described by the mathematical model chosen as a group ( $r = 0.93$  to  $0.99$ , Figure 3.3A), a large intra- or inter-individual difference was observed ( $r = 0.55$  to  $0.98$ ) mainly due to the different recovery conditions. Therefore, recovery kinetics of  $\dot{V}O_2$  was determined by measuring the time required to reach 50%  $\dot{V}O_{2peak}$  (T to 50 $\dot{V}O_{2peak}$ ) in accordance with the previous study (Cohen-Solal et al. 1995). If  $\dot{V}O_2$  did not reach 50%  $\dot{V}O_{2peak}$  during the 4-min recovery (3 out of 84 cases), T to 50 $\dot{V}O_{2peak}$  was defined as 240 sec. However,  $\dot{V}O_2$  reduced to 51%  $\dot{V}O_{2peak}$  during the 4-min recovery in the above all 3 cases, suggesting that this method does not seem to underestimate T to 50 $\dot{V}O_{2peak}$  and is independent of the mathematical model chosen. During each sprint, oxygen uptake ( $ml \cdot min^{-1}$ ) was divided by total work (kJ) in an attempt to determine aerobic contribution relative to mechanical work produced ( $\dot{V}O_2$ -to-sprint ratio) across the sprints and recovery conditions (Buchheit et al. 2012).

#### 2.2.5.3 Study 2

The main aim of measuring cardiorespiratory responses during training sessions was to determine the difference in overall aerobic metabolism between groups (active vs. passive recovery group). Another aim was to examine the influence of sprint interval training on cardiorespiratory responses during repeated sprints. Therefore, oxygen uptake, carbon dioxide production and heart rate were recorded in the first and last training sessions.  $\dot{V}O_2$ ,  $\dot{V}CO_2$  and HR were averaged every 5 sec during the first 4 sprint and recovery intervals, and average values over the 4 sprints and recovery periods were determined.

#### 2.2.5.4 Study 3

This study assessed heart rate responses during two sprint interval training with different sprint duration (15 vs. 30 sec) but the same sprint: rest ratio (1:8). Kavaliauskas et al. (2015)

have recently shown that when sprint duration is kept constant (10 sec), HR is increased with shorter recovery during sprint interval training, resulting in greater aerobic adaptations. However, it has yet to be determined whether sprint duration affects HR responses when sprint: rest ratio is matched. To address this issue, heart rate was recorded in the first, 7<sup>th</sup> and 13<sup>th</sup> training sessions and average values over 4, 5 and 6 sprints were determined, respectively. In addition, HR during the first session was normalised to percentage of total time using a cubic spline method to directly and visually compare heart rate responses between the two training protocols (Figure 5.1).

## 2.2.6 The determination of blood metabolites during repeated sprints

### 2.2.6.1 Study 1

It has been demonstrated that recovery mode does not affect blood lactate accumulation during repeated 30-s Wingate tests despite the difference in repeated sprint performance between recovery conditions (Bogdanis et al. 1996b; Spierer et al. 2004). This suggests that the level of blood lactate may not be a decisive factor in repeated sprint exercise.

Nevertheless, these studies merely compared active or passive but not intensity of recovery, and thus it remains unknown whether the intensity of recovery has an impact on blood lactate metabolism. In addition, although Vincent et al. (2004) reported blood glucose metabolism after a single 30-s Wingate test, no previous studies have investigated effects of recovery mode on glucoregulation during repeated sprints.

Blood samples were taken from participants' fingertips before the first sprint and 180 sec after each sprint to determine blood glucose (Freestyle lite, Abbott Diabetes Care Inc., Alameda, CA) and lactate concentrations (Lactate pro, Arkay Inc., Kyoto, Japan). The skin was punctured using an Accu-check single use lancet (Roche Diagnostics, UK) and pressure

applied to the finger to draw the blood. The initial drop was discarded and the second drop was taken for analysis. The accuracy and reliability of the Lactate pro analyser has been established over the range of 1.0-18.0 mmol·L<sup>-1</sup> (Pyne et al. 2000). Similarly, the suitability and repeatability of the blood glucose test strips have been demonstrated throughout the measuring range of 1.1 to 27.8 mmol·L<sup>-1</sup> (Lock et al. 2011).

#### 2.2.6.2 Study 2

Sprint interval training has been shown to increase enzymatic activities related to anaerobic glycolysis such as phosphofructokinase (PFK) and lactate dehydrogenase (LDH) (Linossier et al. 1997; MacDougall et al. 1998; Dawson et al. 1998; Parra et al. 2000; Rodas et al. 2000). Therefore, changes in blood lactate accumulation with training may partially reflect those in skeletal muscle glycogen metabolism (Rodas et al. 2000). Blood lactate measurement was performed in the first and last training sessions. A finger blood sample was collected at pre-sprint and 180 sec after each sprint until sprint 4 to determine peak and average blood lactate accumulation over the first 4 sprints. Blood sampling procedures were identical to those employed in study 1.

#### 2.2.6.3 Study 3

It has been shown that the majority of anaerobic glycolysis occurs during the initial 15 sec in a maximal sprint (Parolin et al. 1999) and that the contribution from glycolysis to total ATP provision progressively decreases with successive sprint repetitions (Gaitanos et al. 1993; Bogdanis et al. 1996a; Parolin et al. 1999). Therefore, it was assumed that both sprint interval training would result in similar glycogen utilisation despite the difference in sprint duration between the groups (15 vs. 30 sec). To test this assumption, blood lactate analysis was performed during the first training sessions and the level of blood lactate was determined at

pre-sprint and 0, 3, 5, 8 and 10 min after the last sprint (sprint 4) as described in the previous sections.

#### 2.2.7 The selection of resistive force during repeated sprints

All male participants cycled against 7.5% of their bodyweight in accordance with the previous Wingate-based studies (Burgomaster et al. 2005, 2006, 2007, 2008; Gibala et al. 2006; Astorino et al. 2012; Zelt et al. 2014). On the other hand, female participants cycled against 7.5 and 6.5% of their bodyweight in the studies 2 and 3, respectively. The selection of the resistive force for female participants in the study 2 was made based on the previous study showing that training adaptations to Wingate-based HIT are independent of gender with the use of the traditional resistance (i.e. 7.5% bodyweight) in young recreationally active male and female participants (Astorino et al. 2011). However, when combining the data from both training groups in study 2, female participants ( $n = 5$ ) produced a 27% less peak power during training compared to male counterparts ( $n = 9$ ). Considering that the level of peak power produced during training has been related to physiological and performance gains derived from Wingate-based HIT (Hazell et al. 2010), a lighter resistive force might have been more suitable for the female participants to produce a greater power (Billaut and Bishop 2009). Although the female participants studied by Astorino et al. (2011) also showed a lower peak power compared to men, the magnitude of difference between the sexes was less noticeable (19%), possibly due to a lower fat percentage of their female participants (18%) compared with those in study 2 (30%) (Baker and Davies 2006). Consequently, lighter resistance (6.5% of bodyweight) was employed for female participants in the study 3, which resulted in an attenuated decline of peak power (16%) compared to male participants.

### 2.2.8 Crossover point

Crossover point (COP) was determined in an attempt to assess changes in substrate utilisation with training in study 2 and 3. COP represents the work rate at which fuels derived from carbohydrate (CHO) become predominant over those from lipids for energy provision (Brooks and Mercier 1994; Brooks 1997) and therefore it would be an important parameter for substrate utilisation during exercise. Firstly, the rates of CHO and fat oxidation ( $\text{mg} \cdot \text{min}^{-1}$ ) were determined from respiratory gas exchange measures ( $\dot{V}\text{O}_2$ ,  $\dot{V}\text{CO}_2$  and respiratory exchange ratio, RER) according to the equations suggested by Mendelson et al. (2012); CHO oxidation rate =  $1.7012 \times \dot{V}\text{CO}_2 - 3.2255 \times \dot{V}\text{O}_2$ ; and fat oxidation rate =  $1.7 \times (1 - \text{RER}) \times \dot{V}\text{O}_2$ . In order to find crossover point, the rates of CHO and fat oxidation were plotted as a linear function of work rate during the incremental test. The method of elimination was then employed to obtain the intersection point of the two linear lines. The gas exchange measures over the last 1 minute of each completed stage were averaged and a non-completed stage was discarded for this analysis.

### 2.2.9 Critical power

A growing body of evidence suggests that critical power (CP) is an important implication for aerobic function (Jones et al. 2010). CP represents the highest rate of energy production through oxidative phosphorylation that can be sustained without continuously depleting finite energy sources (e.g. PCr) and/or accumulating fatigue-related metabolites (e.g.  $\text{H}^+$ ,  $\text{P}_i$ ) (Burnley et al. 2006; Vanhatalo et al. 2007, 2008; Jones et al. 2010). Therefore, this parameter would provide valuable information regarding exercise tolerance and performance prediction.



CP was determined using a 3-min all-out cycling test on a Monark cycle ergometer (Monark Ergonomic 894E, Varberg, Sweden). All participants cycled against 60W for 3 minutes on the cycle ergometer (Monark Ergonomic 894E, Varberg, Sweden) to warm up. The test then began when the participants reached 110 rpm where resistance was automatically applied (4.5% of bodyweight). They pedalled with an all-out effort for 3 minutes. While strong verbal encouragement was given, no feedback on the elapsed time was provided in an attempt to avoid pacing. Power output was recorded using Monark software (Monark Anaerobic Test Software Version 2.24.2, Monark Exercise AB) and average power output over the final 30 sec was defined as CP. This method has been shown to provide a valid estimation of CP with no difference from the conventionally estimated CP or one derived from a 3-min all-out cycling test on an electronically braked cycle ergometer (Bergstrom et al. 2012). The CP values obtained from the conventional method, the 3-min all-out test on the electronically braked cycle ergometer and Monark ergometer were  $178 \pm 47\text{W}$ ,  $193 \pm 54\text{W}$  and  $186 \pm 44\text{W}$ , respectively and these values were highly inter-correlated ( $r = 0.90 - 0.97$ ) (Bergstrom et al. 2012). In study 2, power produced over each 30-s block (i.e. 6 blocks in total) as well as 3-min total work was also calculated as the integral of power output recorded every second in order to find changes in performance throughout 3 minutes.

#### 2.2.10 Cycling time trial

Time trials have been shown to be a valid and reliable testing protocol to assess endurance performance with coefficient of variation (CV) being  $< 5\%$  (Currell and Jeukendrup 2008). A 10-km cycling time trial test was conducted to bench-mark the effectiveness of training performed in study 2 and 3 with the previous Wingate-based studies reporting performance improvements of between 5 to 10% in 5-km (Hazell et al. 2010), 750-kJ (Gibala et al. 2006) and 250-kJ (Burgomaster et al. 2006, 2007, Babraj et al. 2009) cycling time trials.

After having completed a 3-min warm-up against 60 W on Monark cycle ergometer (Monark Ergomedic 894E, Varberg, Sweden), participants performed a self-paced 10-km cycling time trial against a fixed resistance (2.0kg for males; 1.5kg for females) where they were asked to complete the set distance as fast as possible. Due to the characteristics of Monark cycle ergometer (i.e. friction-braked ergometer), the participants cycled against the fixed resistance and the intensity (power output) of cycling was determined by a self-selected cadence. The lighter resistance was employed for female participants in consideration of the difference in body size between the sexes (Billaut and Bishop 2009). In study 2, a similar cardiorespiratory response was recorded between the sexes during time trials (men vs. women:  $86.7 \pm 10.2$  vs.  $89.7 \pm 9.1$  % $\dot{V}O_{2peak}$ ,  $P = 0.60$ ), indicating that the resistance chosen sufficiently taxed aerobic energy system during the time trials with no difference between the sexes. No information on time, power output and pedal frequency was provided, whereas the amount of distance covered was visible on the screen.

#### 2.2.11 Oxygen pulse

Exercise  $O_2$  pulse is an indicator of stroke volume and arterial-venous  $O_2$  difference (Lavie et al. 2004) and it has been shown to correlate well with the direct measurements of stroke volume using intravascular catheterisation (Whipp et al. 1996; Oliveira et al. 2011). In addition, peak  $O_2$  pulse has been suggested to be a better parameter than  $\dot{V}O_{2peak}$  when predicting chronic heart failure, demonstrating that for every  $1 \text{ ml} \cdot \text{beat}^{-1}$  reduction in  $O_2$  pulse, there was a 24% increase in major clinical events (Lavie et al. 2004). This indicates that oxygen pulse would reflect cardiac function better than peak oxygen uptake. Therefore, oxygen pulse was calculated every 3 weeks (baseline, weeks 3, 6 and 9) in study 3 in order to estimate the time course of cardiac adaptations to Wingate-based HIT. In the incremental test performed every 3 weeks, oxygen pulse at  $\dot{V}O_{2peak}$  was calculated using the following

formula:  $\dot{V}O_2$  (ml·min<sup>-1</sup>) / HR (beats·min<sup>-1</sup>). Moreover, since it has been suggested that  $O_2$  pulse may provide an invalid estimation of stroke volume when body size is not considered into its calculation (Lavie et al. 2004; Oliveira et al. 2011), it was corrected for bodyweight. Accordingly,  $O_2$  pulse (ml·beat<sup>-1</sup>) was divided by weight in kilograms and multiplied by 100 as suggested by Oliveira et al. (2011).

### **2.3 General statistical analyses**

All statistics were run on IBM® SPSS® Version 22.0 for Windows with all data sets being tested for normality using Shapiro-Wilk test. Normality test such as the Shapiro-Wilk test examines whether a sample distribution comes from a population with a normal distribution using the sample mean and variance (Razali and Wah 2011). The normality assumption was assessed via the Shapiro-Wilk test, since previous studies have consistently shown that this test is most powerful among other commonly used normality tests including Kolmogorov-Smirnov, Lilliefors and Anderson-Darling tests (Mendes and Pala 2003; Keskin 2006; Razali and Wah 2011).

To test differences within or between groups, a one- or two-way analysis of variance (ANOVA) with repeated measures was performed, and where appropriate, the test was followed by post-hoc Least significant difference (LSD) test or individuals paired t-tests. In the case of the violation of sphericity, Greenhouse-Geisser corrections were employed. In addition, the normality for a sample distribution derived from the difference between paired scores (i.e. in the case of paired t-tests) was also determined by the Shapiro-Wilk test, and when the normality assumption was violated, a Wilcoxon signed-rank test was used. Where appropriate, Cohen's  $d$  was calculated to quantify the magnitude of difference within or between groups. In the case of a within-subjects factor, it was corrected for dependence between means using the equation suggested by Morris and DeShon (2002);  $d = M_{diff} /$

$SD_{\text{pooled}}\sqrt{2(1 - r)}$ , where  $M_{\text{diff}}$  is mean difference between conditions,  $SD_{\text{pooled}}$  is pooled standard deviation, and  $r$  is correlation between means. Cohen's effect size was defined as follows:  $d < 0.2$  trivial effect, 0.2 - 0.5 small effect, 0.6 - 1.1 moderate effect and 1.2 – 1.9 as a large effect (Cohen 1992). All data are presented as means  $\pm$  standard deviation and the level of significance was set at  $P < 0.05$ .

# **Chapter 3**

## **Study 1**

## **Influence of recovery intensity on oxygen demand, repeated sprint performance and blood metabolites**

### **3.1 Introduction**

Recovery mode (i.e. active vs. passive) has been shown to be important during high-intensity intermittent exercise (Bogdanis et al. 1996b; Dupont et al. 2007; Spencer et al. 2008). When recovery duration is brief (15 to 21 sec) relative to sprint duration (e.g. sprint: rest ratio between 1:1 to 1:5), active recovery results in a greater performance decline in subsequent sprints (Dupont et al. 2007; Spencer et al. 2006; 2008). Furthermore, there was a shorter time to exhaustion during repeated sprints with active recovery regardless of intensity (Dupont et al. 2003; 2004). Conversely, active recovery improves sprint power production when repeated efforts are interspersed with longer recovery periods (180 to 240 sec; sprint: rest ratio between 1:8 to 1:12; Bogdanis et al. 1996b; Connolly et al. 2003; Spierer et al. 2004). This difference could be due to the oxygen cost of active recovery which may inhibit re-oxygenation of the haemoglobin and myoglobin (Dupont et al. 2003, 2004, 2007) or re-synthesis of PCr (Spencer et al. 2006; 2008) in short recoveries. With longer recovery, the increased aerobic metabolism induced by active recovery may allow greater oxygen availability to facilitate PCr restoration during recovery (Bogdanis et al. 1996b; Haseler et al. 1999; McMahon and Jenkins 2002) and increase aerobic energy production during repeated sprints (Dorado et al. 2004).

Over recent years the use of repeated Wingate-based exercise training (four to six all-out 30-s cycling efforts interspersed with 4-min recovery, sprint: rest ratio of 1:8) has become increasingly utilised to improve metabolic functions (e.g. an improved mitochondrial function) and endurance performance (Burgomaster et al. 2005, 2006, 2007, 2008; Gibala et

al. 2006). However, most of the studies have not considered workload during the recovery period. Previously, active recovery (28 to 40% of  $\dot{V}O_{2\max}$ ) during repeated Wingate tests has shown a greater maintenance of power production with similar blood lactate accumulation compared to passive recovery (Bogdanis et al. 1996b; Spierer et al. 2004). Despite this, only Bogdanis et al. (1996b) investigated effects of recovery mode on oxygen uptake during two 30-s Wingate tests interspersed with 4-min recovery. Furthermore, although they found that active recovery increased  $\dot{V}O_2$  during the 4-min recovery compared to passive recovery, only average  $\dot{V}O_2$  was reported due to the method used (Douglas bag method; Bogdanis et al. 1996b). Therefore, the influence of recovery mode on the time course of oxygen uptake recovery has yet to be determined. Moreover, it is unknown whether the intensity of recovery affects overall sprint performance, cardiorespiratory or blood lactate response as the previous studies (Bogdanis et al. 1996b; Spierer et al. 2004; Lopez et al. 2014) merely compared active or passive but not intensity of recovery. In addition, although Vincent et al. (2004) reported blood glucose metabolism after a single 30-s Wingate test, no previous studies have investigated effects of recovery mode on glucoregulation during repeated sprints.

Therefore, this study sought to determine the effects of four different recovery intensities (passive, 20, 30 and 40%  $\dot{V}O_{2\text{peak}}$ ) on oxygen uptake kinetics, sprint performance, and blood lactate and glucose metabolism during repeated 30-s Wingate tests that have been utilised previously to promote training adaptations (Gibala et al. 2006). It was hypothesised that oxygen demand would be increased with recovery intensity, whereas all active recovery intensities would result in improved sprint performance with a similar level of blood lactate and glucose when compared with passive recovery.

## **3.2 Methods and Materials**

### *3.2.1 Participants*

Seven healthy active males who took part in a minimum of 3-h exercise per week participated in the present study (Table 3.1). All participants completed a Physical Activity Readiness Questionnaire (PARQ) to ensure that there were no underlying health issues, and they were fully informed both verbally and in writing about the study before giving their informed consent. The study was approved by the Institutional Ethics Committee and was carried out in line with the Declaration of Helsinki.

### *3.2.2 Experimental design*

All participants were asked to maintain their normal diet and activity throughout the study period and to refrain from alcohol intake and any form of intense physical activity for 24 h prior to each session. Participants reported to the Human Performance Laboratory after a 4-h fast on each occasion. All participants performed 5 sessions at a similar time of day ( $\pm 2$ h) in a controlled environment throughout the study period. Each session was separated by at least a period of 48h but by no more than 2 weeks. On the initial visit, body composition was recorded on a calibrated bioelectrical impedance meter (SC-330ST Tanita Body Composition Analyser, Tanita Europe BV, Amsterdam, Netherlands) where body fat and mass were recorded (Table 3.1).



**Table 3.1** Physical and Physiological characteristics of the participants.

Variables	Participants (n = 7)
Age (year)	23 ± 5
Height (cm)	180 ± 6
Body mass (kg)	74 ± 8
Body fat (%)	14.4 ± 4.9
$\dot{V}O_{2peak}$ (l·min <sup>-1</sup> )	3.6 ± 0.6
$\dot{V}O_{2peak}$ (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )	48.1 ± 5.1
Pmax (watts)	321 ± 71
HR max (beats·min <sup>-1</sup> )	181 ± 11

Values are means ± SD.  $\dot{V}O_{2peak}$ , peak oxygen uptake; Pmax, maximal power output; HRmax, maximal heart rate.

### 3.2.3 Determination of $\dot{V}O_{2peak}$ , maximal heart rate and maximal power output

On the initial visit, maximal values ( $\dot{V}O_{2peak}$ , HRmax and Pmax) were determined as described in the section 2.2.3.1 and those values are shown in the table 3.1.

### 3.2.4 Determination of effects of recovery intensity on performance, cardiorespiratory responses and blood metabolites during repeated sprints

On the 2 to 5 visits, all participants performed 4 x 30-s cycle sprints against 7.5% bodyweight interspersed with 4-min recovery at a given recovery intensity (passive, 20, 30 or 40% $\dot{V}O_{2peak}$ ) on each day, and effects of recovery intensity on repeated sprint performance (section 2.2.4.2) and cardiorespiratory (section 2.2.5.2) and blood responses (section 2.2.6.1) were determined as explained in chapter 2.

### 3.2.5 Statistical Analyses

All data are presented as means  $\pm$  standard deviation. Before conducting parametric tests, the Shapiro-Wilk test was performed to ensure that all values were normally distributed. A two-way analysis of variance (ANOVA) with repeated measures was used to determine overall differences between recovery conditions (passive, 20, 30 and 40%  $\dot{V}O_{2peak}$ ) and sprint/recovery number for Wingate performance, cardiorespiratory and blood lactate and glucose variables. Greenhouse-Geisser corrections were used where the violation of sphericity was detected. In the case of a significant main effect of condition, the test was followed by post-hoc Least significant difference (LSD) test. Where a significant sprint/recovery number by condition interaction effect was observed, one-way repeated ANOVA with post-hoc LSD test was performed to determine differences among the conditions for each sprint/recovery. Moreover, where the post-hoc test revealed a significant difference between conditions, effect size (Cohen's  $d$ ) was calculated. Due to the study design (i.e. repeated measures), Cohen's  $d$  was corrected for dependence between means using the equation suggested by Morris and DeShon (2002);  $d = M_{diff} / SD_{pooled} \sqrt{2(1 - r)}$ , where  $M_{diff}$  is mean difference between conditions,  $SD_{pooled}$  is pooled standard deviation, and  $r$  is correlation between means. Cohen's effect size was defined as follows:  $d < 0.2$  trivial effect, 0.2 - 0.5 small effect, 0.6 - 1.1 moderate effect and 1.2 – 1.9 as a large effect (Cohen 1992). Changes over time ( $P < 0.01$  in all cases) are only mentioned where appropriate for clarity. All statistics were run on IBM® SPSS® Version 21.0 for Windows and the significance level was set at  $P < 0.05$ .

### 3.3 Results

#### 3.3.1 Wingate performance

There was no main effect of recovery condition in the overall peak power, average power or power drop rate (Table 3.2). However, a significant sprint by condition interaction effect was observed in peak power, and the drop rates of both peak and average power (Table 3.2).

Although the 30% recovery condition temporarily decreased peak power compared to the 20% recovery during sprint 2 ( $P < 0.01$ ,  $d = 1.44$ ), all active recovery conditions improved it during the last sprint compared with the passive recovery ( $P < 0.05$  in all cases,  $d = 0.94$ , 1.01 and 0.95 for passive vs. 20, 30 and 40%, respectively) (Table 3.2). During sprint 2, a greater drop rate in peak power was observed in the 40% recovery condition compared to the 20% recovery ( $P < 0.05$ ,  $d = 0.99$ ) or the passive recovery ( $P < 0.05$ ,  $d = 1.17$ ), whereas that in average power was significantly improved by the 30 and 40% recovery conditions compared with the passive recovery ( $P < 0.05$  in both cases,  $d = 1.46$  and 1.18 for passive vs. 30 and 40%, respectively), or for the 40% recovery compared to the 20% recovery ( $P < 0.05$ ,  $d = 1.17$ ) during the last sprint (Table 3.2).

**Table 3.2** Peak power, average power and respective power drop rate across the sprints and recovery conditions

Performance parameters	Recovery conditions			
Peak power ( $\text{W} \cdot \text{kg}^{-1}$ ) <sup>#</sup>	Passive	20%	30%	40%
Sprint 1	12.6 ± 2.2	12.8 ± 2.1	12.4 ± 1.9	13.1 ± 1.7
Sprint 2 <sup>§</sup>	11.7 ± 2.1	12.1 ± 2.1 <sup>††</sup>	10.8 ± 2.0	11.4 ± 1.4
Sprint 3	10.4 ± 1.9	10.9 ± 2.0	10.0 ± 1.5	11.0 ± 1.6
Sprint 4 <sup>§</sup>	9.5 ± 1.7	10.1 ± 1.8*	10.3 ± 1.8*	10.6 ± 1.8*
PP drop relative to Sprint 1 (%) <sup>##</sup>	Passive	20%	30%	40%
Sprint 2 <sup>§</sup>	7.4 ± 5.4	5.8 ± 7.9	12.7 ± 7.4	12.7 ± 5.5* <sup>†</sup>
Sprint 3	17.0 ± 9.9	14.8 ± 7.2	19.5 ± 3.4	16.0 ± 5.8
Sprint 4	23.7 ± 11.8	20.9 ± 10.3	16.6 ± 8.5	19.3 ± 10.0
Average power ( $\text{W} \cdot \text{kg}^{-1}$ )	Passive	20%	30%	40%
Sprint 1	8.3 ± 0.5	8.4 ± 0.5	8.3 ± 0.5	8.3 ± 0.4
Sprint 2	7.3 ± 0.7	7.6 ± 0.7	7.3 ± 0.9	7.3 ± 0.5
Sprint 3	6.8 ± 0.9	7.0 ± 0.6	6.7 ± 0.6	7.0 ± 0.3
Sprint 4	6.4 ± 0.8	6.8 ± 0.7	6.8 ± 0.6	6.9 ± 0.5
AP drop relative to Sprint 1 (%) <sup>#</sup>	Passive	20%	30%	40%
Sprint 2	11.2 ± 5.0	10.4 ± 5.2	11.6 ± 7.9	12.0 ± 4.9
Sprint 3	17.5 ± 9.3	16.8 ± 4.5	18.5 ± 6.3	15.7 ± 5.6
Sprint 4 <sup>§</sup>	22.4 ± 8.9	19.9 ± 6.1	18.4 ± 7.3*	16.6 ± 6.2* <sup>†</sup>

Values are means ± SD. PP, peak power; AP, average power. <sup>##</sup>Indicates sprint by condition interaction effect (P<0.01). <sup>#</sup>Indicates sprint by condition interaction effect (P<0.05). <sup>§</sup>Indicates main effect of condition for each sprint (P<0.05). \*Indicates P<0.05 vs. passive recovery. <sup>†</sup>Indicates P<0.05 vs. 20%. <sup>††</sup>Indicates P<0.01 vs. 30%. Sprint by condition interaction effect was detected by a 2-way analysis of variance with repeated measures, while a one-way ANOVA with post-hoc LSD test was employed to determine the difference between recovery conditions for each sprint.

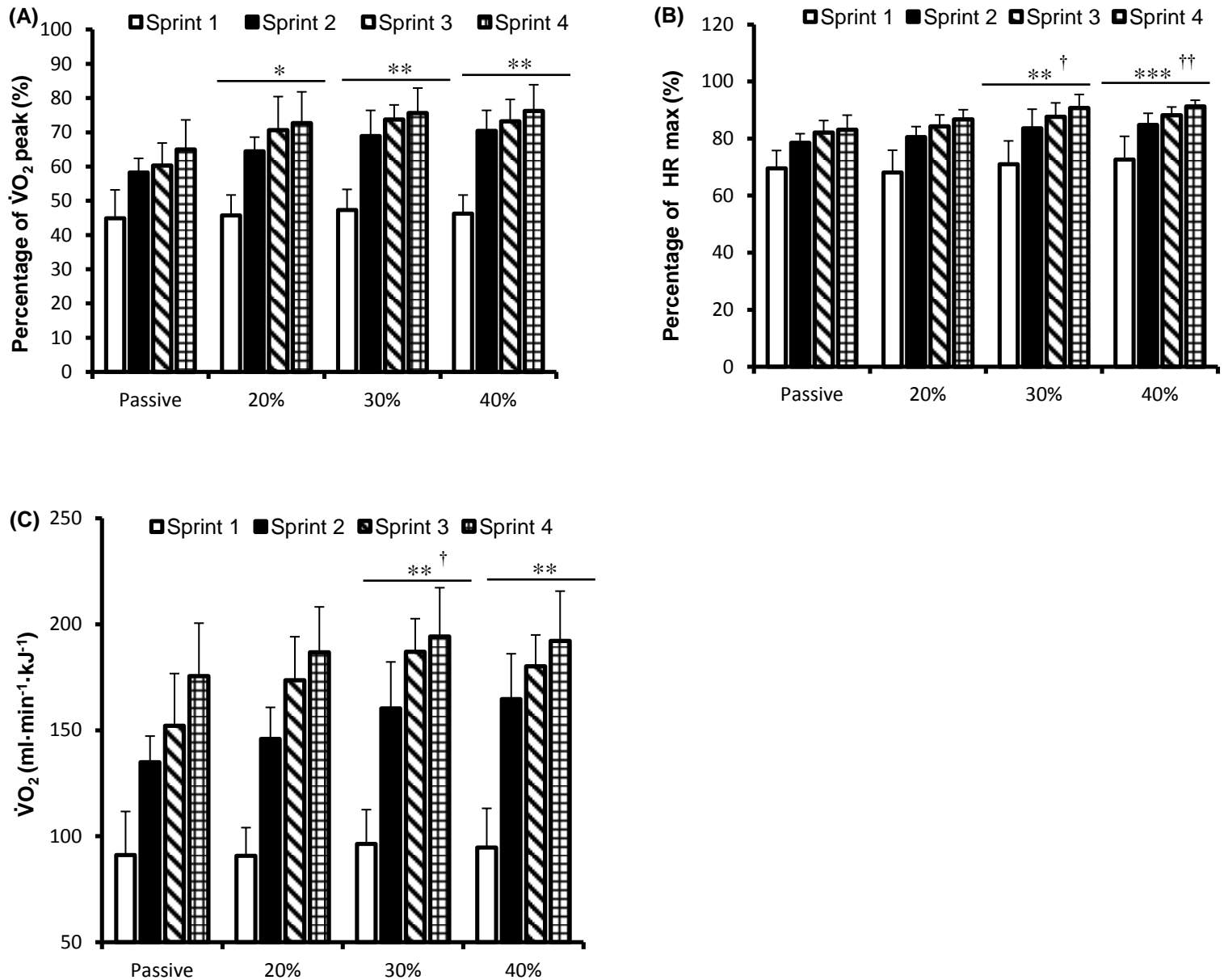
### 3.3.2 Oxygen uptake during sprints

There was a main effect of recovery condition in average  $\dot{V}O_2$  during sprints (Figure 3.1A: Passive:  $57 \pm 5$ ; 20%:  $63 \pm 6$ ; 30%:  $66 \pm 5$ ; 40%:  $67 \pm 5$  %  $\dot{V}O_{2peak}$ ,  $P < 0.001$ ). Sprint-averaged  $\dot{V}O_2$  was significantly elevated for all active recovery conditions compared with the passive recovery (Passive vs. 20%:  $P < 0.05$ ,  $d = 1.16$ ; Passive vs. 30 and 40%:  $P < 0.01$ ,  $d = 2.12$  and  $2.33$  for passive vs. 30 and 40%, respectively), while there was no significant difference among active recovery conditions (Figure 3.1A). There was a main effect of condition in  $\dot{V}O_2$ -to- sprint work ratio (Figure 3.1C: Passive:  $138 \pm 17$ ; 20%:  $149 \pm 14$ ; 30%:  $159 \pm 15$ ; 40%:  $158 \pm 17$   $ml \cdot min^{-1} \cdot kJ^{-1}$ ,  $P = 0.001$ ).  $\dot{V}O_2$ -to- sprint work ratio was significantly increased in the 30 and 40% recovery groups compared to the passive recovery ( $P < 0.01$  in both cases,  $d = 1.64$  and  $2.23$  for passive vs. 30 and 40%, respectively). It was also significantly elevated for the 30% recovery condition compared with the 20% recovery condition ( $P < 0.05$ ,  $d = 1.16$ ) (Figure 3.1C).

### 3.3.3 Heart rate during sprints

There was a main effect of recovery condition in average heart rate during sprints (Figure 3.1B: Passive:  $78 \pm 4$ ; 20%:  $80 \pm 4$ ; 30%:  $83 \pm 6$ ; 40%:  $84 \pm 4$  % HR max,  $P < 0.001$ ). Sprint-averaged HR was significantly elevated for the 30 and 40% recovery groups compared with the passive recovery (Passive vs. 30%:  $P < 0.01$ ,  $d = 2.74$ ; Passive vs. 40%:  $P < 0.001$ ,  $d = 4.04$ ) or 20% recovery group (20% vs. 30%:  $P < 0.05$ ,  $d = 1.14$ ; 20% vs. 40%:  $P < 0.01$ ,  $d = 2.74$ ), but there was no significant difference between the 20% recovery and the passive recovery (Figure 3.1B).

**Figure 3.1** Percentage of  $\dot{V}O_{2peak}$  (A), percentage of HR max (B) and  $\dot{V}O_2$  –to- sprint work ratio (C) across sprints



\*\*\*Indicates  $P < 0.001$  vs. passive recovery. \*\*Indicates  $P < 0.01$  vs. passive recovery. \*Indicates  $P < 0.05$  vs. passive recovery.  $\dagger\dagger$  Indicates  $P < 0.01$  vs. 20%.  $\dagger$  Indicates  $P < 0.05$  vs. 20%. Significant differences across the recovery conditions are only shown for clarity. The main effect for condition was detected by 2-way repeated ANOVA with the difference between each of recovery conditions being determined by the post-hoc LSD test.

### 3.3.4 Oxygen uptake during recovery

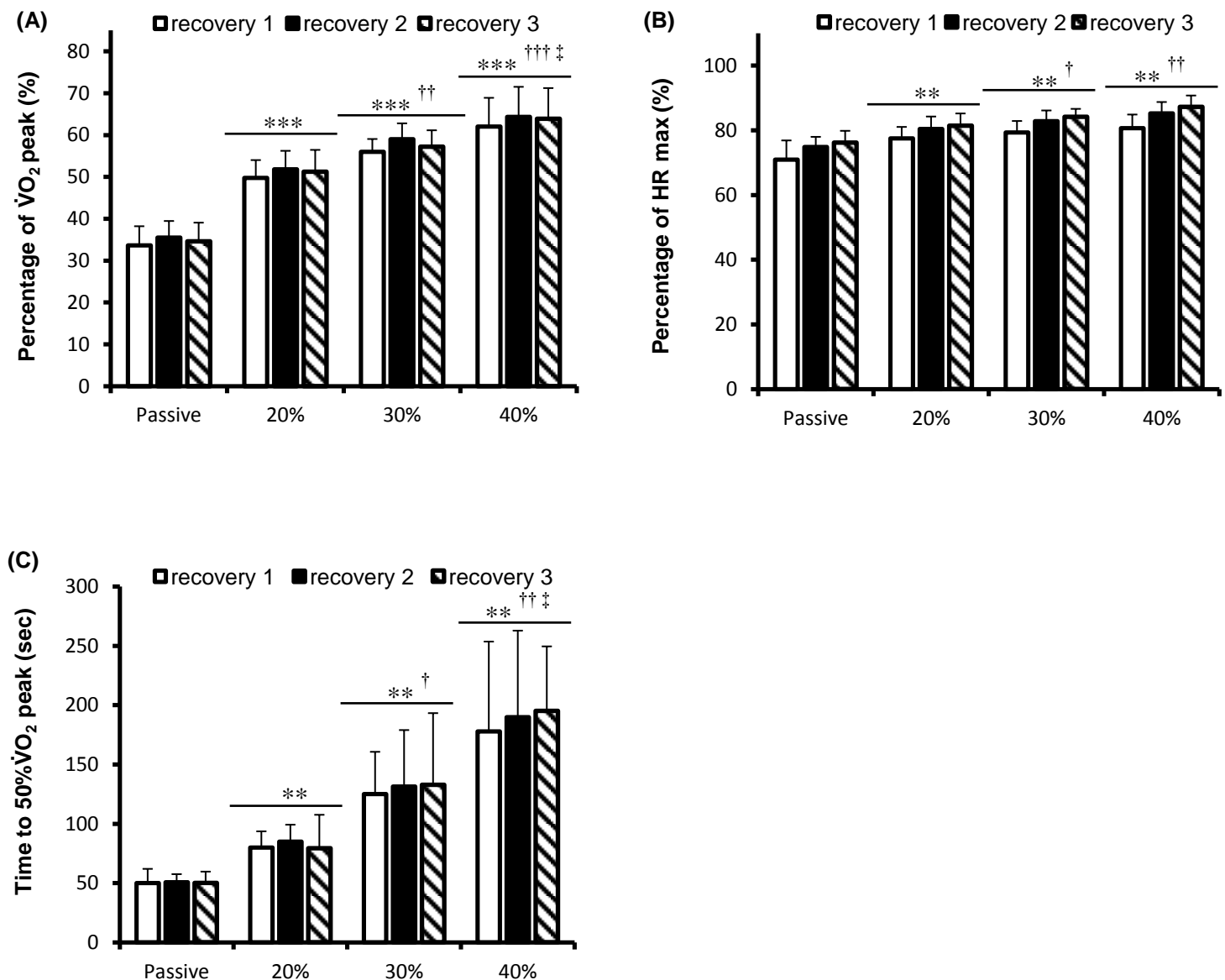
There was a main effect of recovery condition in average  $\dot{V}O_2$  during recovery (Figure 3.2A: Passive:  $35 \pm 4$ ; 20%:  $51 \pm 5$ ; 30%:  $57 \pm 4$ ; 40%:  $63 \pm 7$  % $\dot{V}O_{2peak}$ ,  $P < 0.001$ ). Recovery-averaged  $\dot{V}O_2$  was significantly increased for all active recovery conditions compared with the passive recovery ( $P < 0.001$  in all cases,  $d = 4.52, 6.49$  and  $6.61$  for passive vs. 20, 30 and 40%, respectively) (Figure 3.2A). It was also significantly elevated for the 30 and 40% recovery groups compared with the 20% recovery group (20% vs. 30%:  $P < 0.01$ ,  $d = 2.22$ ; 20% vs. 40%:  $P < 0.001$ ,  $d = 5.66$ ), and for the 40% recovery compared to the 30% recovery condition ( $P < 0.05$ ,  $d = 1.96$ ) (Figure 3.2A). Likewise there was a main effect of condition in time required to reach 50%  $\dot{V}O_{2peak}$  (Figure 3.2C: Passive:  $50 \pm 9$ ; 20%:  $81 \pm 17$ ; 30%:  $130 \pm 43$ ; 40%:  $188 \pm 62$  sec,  $P < 0.001$ ). T to 50% $\dot{V}O_{2peak}$  was increased for all active recovery conditions compared with the passive recovery ( $P < 0.01$  in all cases,  $d = 2.22, 2.53$  and  $2.99$  for passive vs. 20, 30 and 40%, respectively) (Figure 3.2C). It was also significantly elevated for the 30 and 40% recovery groups compared with the 20% recovery group (20% vs. 30%:  $P < 0.05$ ,  $d = 1.69$ ; 20% vs. 40%:  $P < 0.01$ ,  $d = 2.88$ ), and for the 40% recovery compared to the 30% recovery condition ( $P < 0.05$ ,  $d = 1.53$ ) (Figure 3. 2C). Example of recovery kinetics of  $\dot{V}O_2$  is shown in Figure 3.3A.

### 3.3.5 Heart rate during recovery

There was a main effect of recovery condition in average HR during recovery (Figure 3.2B: Passive:  $74 \pm 4$ ; 20%:  $80 \pm 4$ ; 30%:  $82 \pm 3$ ; 40%:  $84 \pm 4$  %HR max,  $P < 0.001$ ). Recovery-averaged HR was significantly increased for all active recovery conditions compared with the passive recovery ( $P < 0.01$  in all cases,  $d = 2.15, 7.36$  and  $2.73$  for passive vs. 20, 30 and 40%, respectively) (Figure 3.2B). It was also significantly elevated for the 30 and 40% recovery

groups compared with the 20% recovery group (20% vs. 30%:  $P < 0.05$ ,  $d = 0.96$ ; 20% vs. 40%:  $P < 0.01$ ,  $d = 1.62$ ) (Figure 3.2B). Example of recovery kinetics of HR is shown in Figure 3.3B.

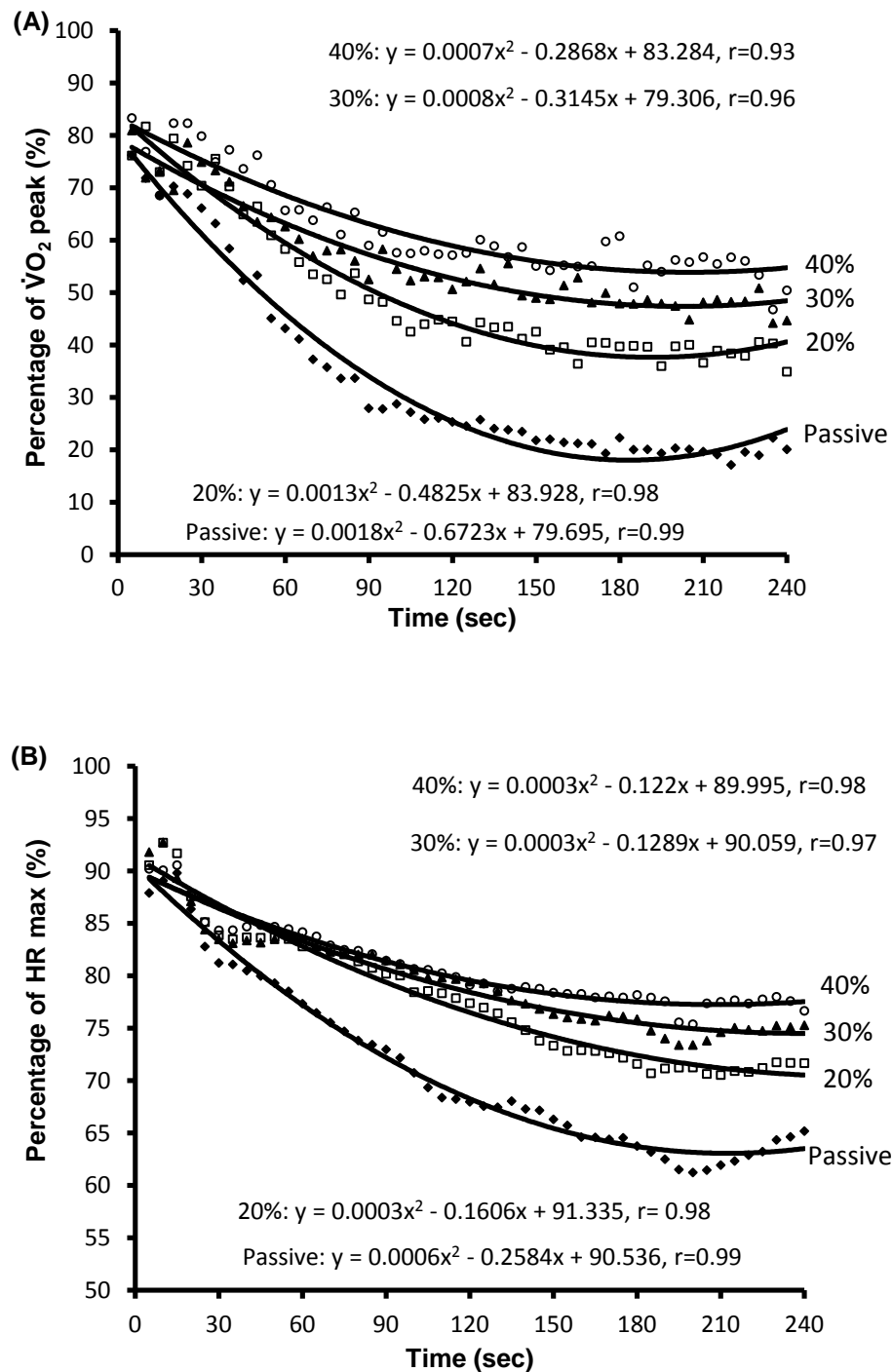
**Figure 3.2** Percentage of  $\dot{V}O_{2peak}$  (A), percentage of HR max (B) and time required to reach 50%  $\dot{V}O_{2peak}$  (C) during recovery periods



\*\*\*Indicates  $P < 0.001$  vs. passive recovery. \*\*Indicates  $P < 0.01$  vs. passive recovery. †††Indicates  $P < 0.001$  vs. 20%. ††Indicates  $P < 0.01$  vs. 20%. †Indicates  $P < 0.05$  vs. 20%. ‡Indicates  $P < 0.05$  vs. 30%. Significant differences across the recovery conditions are only shown for clarity. Statistical analyses were performed as described in the legend of figure 3.1.



**Figure 3.3** Example of recovery kinetics of oxygen uptake (A) and HR (B) during recovery  
(group mean)



Error bars are not shown for clarity

### 3.3. 6 Blood metabolites

There was no main effect for condition or sprint by condition interaction effect either in blood lactate or glucose concentration (Table 3.3). Although blood lactate level significantly rose with repeated sprints, the level of blood glucose was unchanged throughout the sprint protocol (Table 3.3).

**Table 3.3** Blood lactate and glucose concentrations across the sprints and recovery conditions

Blood metabolites	Recovery conditions			
Blood lactate (mmol·l <sup>-1</sup> )	Passive	20%	30%	40%
Pre-Sprint	1.6 ± 0.5	1.7 ± 0.5	1.5 ± 0.4	1.9 ± 0.3
Post-Sprint 1 at 180 sec***	10.7 ± 0.9	12.1 ± 2.1	11.3 ± 1.2	12.2 ± 1.9
Post-Sprint 2 at 180 sec***†††	14.0 ± 0.7	14.6 ± 2.5	14.6 ± 1.9	13.9 ± 1.1
Post-Sprint 3 at 180 sec***†††	14.8 ± 0.8	15.8 ± 2.0	14.7 ± 2.9	15.3 ± 1.7
Post-Sprint 4 at 180 sec***†††‡	15.0 ± 1.6	14.9 ± 0.7	15.8 ± 1.2	15.7 ± 1.6
Blood glucose (mmol·l <sup>-1</sup> )	Passive	20%	30%	40%
Pre-Sprint	4.5 ± 0.5	5.3 ± 0.5	5.4 ± 1.3	4.6 ± 0.3
Post-Sprint 1 at 180 sec	4.7 ± 0.3	5.2 ± 0.3	5.4 ± 1.1	4.9 ± 0.3
Post-Sprint 2 at 180 sec	5.0 ± 0.4	5.2 ± 0.7	5.2 ± 1.0	4.7 ± 0.4
Post-Sprint 3 at 180 sec	5.0 ± 0.5	5.2 ± 0.8	5.2 ± 1.0	5.0 ± 0.7
Post-Sprint 4 at 180 sec	5.0 ± 0.6	5.4 ± 0.8	5.4 ± 0.8	5.5 ± 0.5

Values are means ± SD. \*\*\*Indicates P < 0.001 vs. pre-sprint. †††Indicates P < 0.001 vs. post-sprint 1. ‡Indicates P < 0.05 vs. post-sprint 2. The main effect for sprint number in blood lactate concentration was detected via 2-way repeated ANOVA with the difference between each of sprint number being determined by the post-hoc LSD test.

### 3.4 Discussion

This study sought to determine the effects of recovery intensity on the oxygen uptake kinetics, repeated 30-s Wingate performance and blood lactate and glucose metabolism. To the best of my knowledge, this is the first study to examine the effects of four different recovery intensities (passive, 20, 30 and 40% $\dot{V}O_{2peak}$ ) during the typical Wingate-based exercise training protocols (Gibala et al. 2006). The novel findings of the study are that oxygen cost during recovery is increased with the intensity of recovery, aerobic contribution to repeated sprint performance is only elevated by the higher recovery intensities and any active recovery intensity does not cause an alteration of blood lactate or glucose accumulation when compared with the passive recovery.

#### *3.4.1 The influence of recovery intensity on cardiorespiratory responses and sprint performance*

Although average  $\dot{V}O_2$  during the repeated sprints was increased in all active recovery conditions compared with the passive recovery,  $\dot{V}O_2$ - to- sprint work ratio was only significantly increased by the 30 and 40% recovery groups (Figure 3.1A & 3.1C).  $\dot{V}O_2$  at the end of the recovery periods was greater in the higher recovery conditions (30%:  $46.5 \pm 2.7$ ; 40%:  $47.6 \pm 6.5$  % $\dot{V}O_{2peak}$ ) compared to the 20% recovery group ( $38.1 \pm 1.7$  % $\dot{V}O_{2peak}$ ,  $P < 0.01$ ) as well as the passive recovery ( $21.9 \pm 4.6$  % $\dot{V}O_{2peak}$ ,  $P < 0.001$ ; Figure 3.3A), suggesting that the participants began sprints with an elevated oxidative metabolism following the higher recovery intensities compared to the passive or 20% recovery. Furthermore, HR during the sprints was significantly increased by the higher recovery intensities compared to the passive or 20% recovery condition (Figure 3.1B), indicating an increased muscle blood flow and thus a greater  $O_2$  delivery to the working muscles (Tordi et al. 2003) during the sprints in these conditions. The elevated whole body  $\dot{V}O_2$  along with the

increased HR following the higher recovery intensities seems to have become increasingly important with the successive sprint repetitions where muscle O<sub>2</sub> extraction and thus aerobic contribution to mechanical work progressively increase (Figure 3.1C; Jones et al. 2003; Buchheit et al. 2012). The attenuated drop in average power induced by the higher recovery intensities during the last sprint (Table 3.2) would support this assumption.

Conversely, there was a tendency for the 30 and 40% recovery groups to cause greater peak power decline during sprint 2 compared with the passive or the lower recovery condition (Table 3.2). In contrast to the current study, Bogdanis et al. (1996b) demonstrated that active recovery at 40%  $\dot{V}O_{2max}$  increased power production in sprint 2 compared to passive recovery, which was totally attributed to a 3.1% higher power output produced during the initial 10s, when two 30-s cycle sprints were separated by 4 min. Since they also found a high correlation between re-synthesis of PCr and recovery of power output during the initial 10s of the second 30-s sprint ( $r = 0.84$ ,  $P < 0.05$ ; Bogdanis et al. 1996a), the improved power production might be attributed to a greater O<sub>2</sub> availability for PCr re-synthesis induced by the active recovery (as reflected by greater  $\dot{V}O_2$  compared with passive recovery,  $P < 0.01$ ) during the 4-min recovery (Bogdanis et al. 1996b). In support of this, Haseler et al. (1999) demonstrated that hyperoxia caused by greater fractions of inspired O<sub>2</sub> enhanced PCr restoration during 5-min recovery following submaximal exercise. Nevertheless, considering that a close relationship has been shown between time course of  $\dot{V}O_2$  recovery and that of PCr restoration (Cohen-Solal et al. 1995; McMahon and Jenkins 2002), the greater decrease in peak power seen in the higher recovery conditions during sprint 2 could be explained by the prolonged  $\dot{V}O_2$  recovery (Figure 3.2C). Indeed, it has been demonstrated that a prolonged T to 50 $\dot{V}O_{2peak}$  results in a slower rate of PCr recovery (Cohen-Solal et al. 1995) and T to 50 $\dot{V}O_{2peak}$  is greater with higher intensity recoveries in the current study (Figure 3.2C).

Although Bogdanis et al. (1996b) only reported average  $\dot{V}O_2$  during the 4-min recovery (55%  $\dot{V}O_{2max}$ ) and thus the time course of  $\dot{V}O_2$  recovery is unknown, a greater maximal aerobic capacity ( $4.28 \pm 0.13 \text{ l}\cdot\text{min}^{-1}$ ; approximately  $55 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) of their participants compared to the current study ( $3.6 \pm 0.6 \text{ l}\cdot\text{min}^{-1}$ , or  $48.1 \pm 5.1 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) might have allowed faster  $\dot{V}O_2$  recovery (Tomlin and Wenger 2001; Dupont et al. 2010) possibly resulting in faster and/or greater PCr restoration. Similar to the current study, Lopez et al. (2014) employed six 30-s cycle sprints alternated by 4-min recovery, and saw a greater peak power drop during sprint 2 in active recovery condition compared with passive recovery, while the active recovery improved average power during sprint 5 and 6. Although they did not report  $\dot{V}O_{2max}/\dot{V}O_{2peak}$  of their participants and therefore their findings cannot be directly compared with those of the current study or the study by Bogdanis et al. (1996b), it could be assumed that active recovery may not be beneficial when only two sprints are performed whereas it would facilitate maintaining power production with the sprint repetitions, as the sprints become more aerobically demanding (Gaitanos et al. 1993; Bogdanis et al. 1996a; Parolin et al. 1999; Buchheit et al. 2012). Lopez et al. (2014) also found that the active recovery tended to improve peak power during sprint 5 and 6 compared to the passive recovery (improved by  $0.3 \text{ W}\cdot\text{kg}^{-1}$  in both cases), which is in agreement with the current study (i.e. peak power during the last sprint; Table 3.2). This may suggest that greater aerobic metabolism caused by active recovery during the rest periods may become increasingly beneficial to PCr restoration as sprints are repeated (Tomlin and Wenger 2001) where muscle re-oxygenation rate progressively increases (Buchheit et al. 2012). Changes in muscle metabolites such as PCr,  $P_i$  and pH during high-intensity cycling have been determined via  $^{31}\text{P}$  magnetic resonance spectroscopy (MRS) (Jones et al. 2008; Vanhatalo et al. 2010), whereas muscle oxygenation state (the rates of muscle de-oxygenation and re-oxygenation) during Wingate-based HIT has been derived from near infrared spectroscopy (NIRS) (Buchheit et al. 2012). Therefore, a

future study could directly determine the effects of recovery intensity on muscle metabolites and muscle oxygenation state during repeated sprint exercise using these measurement (MRS and NIRS).

#### *3.4.2 The influence of recovery intensity on blood lactate and glucose metabolism*

The blood lactate level markedly rose after sprint 1, however the magnitude of increase in blood lactate notably decreased with the successive bouts (Table 3.3), indicating that anaerobic glycolysis progressively reduced with repeated sprints (Gaitanos et al. 1993; Bogdanis et al. 1996a; Parolin et al. 1999). The present study did not find any difference in blood lactate concentration across the recovery intensities. This is not in line with the previous studies employing longer recovery periods (> 450 sec) where active recovery promoted a greater clearance of lactate from the blood (Spierer et al. 2004; Wahl et al. 2013) but similar to those employing shorter recovery periods (15 to 240 sec) where recovery mode did not affect blood lactate concentration despite the difference in repeated sprint performance between recovery conditions (Bogdanis et al. 1996b; Connolly et al. 2003; Dupont et al. 2003, 2004). This indicates that the level of blood lactate may not be a decisive factor in repeated sprint exercise and longer recovery duration might be needed to see effects of an increased blood flow induced by active recovery on lactate transport and uptake by other tissues (Belcastro and Bonen 1975; Brooks 2000; Gladden 2000).

Blood glucose remained unchanged with time and there was no difference among the recovery conditions (Table 3.3). During intense exercise hepatic glucose production (rate of appearance, Ra) has been shown to be mainly mediated by catecholamine response (Marliss et al. 2000; Sigal et al. 2000; Vincent et al. 2004), whereas skeletal muscle glucose uptake

(rate of disappearance,  $R_d$ ) is attributed to several factors such as metabolic stress and the degree of glycogen depletion (Hayashi et al. 1998; Richter et al. 2001; Helge et al. 2003). Similar to Vincent et al. (2004) who observed no increase in blood glucose after a 30-s sprint in healthy men, we did not observe significant difference in blood glucose concentration across the sprints as well as recovery conditions. Greer et al. (1998) demonstrated a continuous rise in plasma epinephrine and norepinephrine following repeated Wingate tests, suggesting a greater rate of hepatic glucose production to maintain blood glucose levels either via elevated gluconeogenesis or glycogenolysis (Sherwin and Saccá 1984). This suggests that similar hormonal and/or metabolic response as well as the degree of muscular work (i.e. glycogen depletion) was achieved among the recovery conditions in the present study.

### **3.5 Conclusions**

The present study demonstrates that  $\dot{V}O_2$  recovery kinetics and aerobic contribution to power generation during repeated Wingate tests are dependent on the intensity of recovery while no difference was observed in blood lactate or glucose concentration among the recovery conditions. Peak power was decreased with the higher recovery intensities (30 and 40%  $\dot{V}O_{2peak}$ ) compared with the passive recovery or light recovery intensity (20%  $\dot{V}O_{2peak}$ ) during sprint 2, and this might reflect an impaired intramuscular recovery (e.g. PCr recovery) due to the prolonged  $\dot{V}O_2$  recovery induced by those conditions. On the other hand, aerobic contribution to sprint performance was only increased by the higher intensities (by ~15%), which likely resulted in the less decreased average power during the last sprint. Furthermore, all active recovery intensities resulted in ~ 6 to 12% greater peak power when compared with the passive recovery during the last sprint with the magnitude of improvements being increased with the intensity of recovery. Taken together, a higher recovery intensity ( $\geq 30\% \dot{V}O_{2peak}$ ) is recommended when performing typical Wingate-based HIT (4 to 6 30-s

sprints interspersed with 4 min of recovery) where aerobic contribution to sprint performance progressively increases with successive bouts. The higher recovery intensity would ensure an increased aerobic demand (e.g.  $\dot{V}O_2$ , HR) during the training with sprint performance (the level of power production) being improved with successive sprint repetitions.



# **Chapter 4**

## **Study 2**

## **Effects of recovery mode on physiological and performance adaptations to sprint interval training over 2 weeks**

### **4.1 Introduction**

It has been established that a 2-week Wingate-based high intensity training programme (HIT) consisting of 4 to 6 x 30-s maximal efforts with 4-min recovery (1:8 work to rest ratio) can induce various training adaptations such as improvements in mitochondrial function, muscle buffering capacity and exercise performance (e.g. time trial, time to exhaustion) (Burgomaster et al. 2005, 2006; Gibala et al. 2006). In contrast, there has been mixed findings on improvements in  $\dot{V}O_{2\max}$  following 2 weeks of Wingate-based HIT with some studies reporting no change (Burgomaster et al. 2005, 2006) and others reporting an increase of approximately 9% (Bailey et al. 2009; Hazell et al. 2010; Astorino et al. 2012). However, increases in  $\dot{V}O_{2\max}$  are more robustly seen after a longer training intervention (4 to 6 weeks) (Burgomaster et al. 2008; Trilk et al. 2011; Macpherson et al. 2011; Zelt et al. 2014). This suggests that Wingate-based HIT could be a time-efficient strategy to induce cardiorespiratory as well as skeletal muscle adaptations rapidly. Whilst an improvement in endurance performance has been consistently reported after Wingate-based HIT over 2 to 6 weeks (Gibala et al. 2006; Burgomaster et al. 2005, 2006, 2007; Babraj et al. 2009; Hazell et al. 2010; Macpherson et al. 2011), limited data is available to determine what aspects of endurance have been altered since all of the above Wingate-based studies assessed performance via either time-trial or time to exhaustion. Recently, Zelt et al. (2014) have demonstrated a 5 to 7% improvement in critical power using a 3-min all-out cycling test following 12 sessions of Wingate-based HIT over 4 weeks. This suggests that the changes in mitochondrial activity result in an increased ability to maintain oxidative power production at a higher level after Wingate-based HIT.

Despite these training benefits, most of studies have not considered workload during the recovery period. Indeed, passive recovery or very light cycling ( $< 30\text{W}$ ) (Burgomaster et al. 2005, 2006; Gibala et al. 2006; Bailey et al. 2009) or unloaded cycling (Hazell et al. 2010; Astorino et al. 2012; Zelt et al. 2014) are commonly employed in the Wingate-based HIT studies. However, active recovery (cycling at 28 - 40% of  $\dot{V}\text{O}_{2\text{max}}$ ) has been shown to facilitate maintenance of power production during repeated 30-s Wingate tests compared to passive recovery (Bogdanis et al. 1996b; Spierer et al. 2004; Lopez et al. 2014) and provides an elevated cardiorespiratory demand (e.g. increased HR and  $\dot{V}\text{O}_2$ ) (Bogdanis et al. 1996b). When rest intervals are kept short during HIT protocols consisting of 4 to 6 x 10-s cycling sprints, it produces a greater cardiorespiratory demand which results in greater endurance adaptations (Kavaliuskas et al. 2015). Likewise, active recovery (50% of maximal aerobic velocity, MAV) has been shown to induce a greater cardiorespiratory demand, as assessed by time spent above 90%  $\dot{V}\text{O}_{2\text{max}}$ , compared to passive recovery during HIT consisting of repeated 30-s runs at 105% MAV, resulting in greater cardiorespiratory improvements (Ben Abderrahman et al. 2013). Thus, active recovery at low to moderate intensity ( $\sim 40\%\dot{V}\text{O}_{2\text{max}}$ ) may induce greater endurance adaptations when performing Wingate-based HIT.

Therefore, this study aimed to determine effects of recovery mode (i.e. passive vs. active) on endurance capacity during Wingate-based HIT over 2 weeks. It was hypothesised that active recovery would induce greater endurance adaptations when compared with passive recovery due to the higher metabolic demand during the training.

## 4.2 Methods and Materials

### 4.2.1 Participants

Fourteen healthy active male and female participants (M: 9; F: 5) who took part in a minimum of 3-h exercise per week participated in this study (Table 3.1). All were physically active but none of them were participating in regular sporting competitions during the study period. All participants completed a Physical Activity Readiness Questionnaire (PARQ) to ensure there were no underlying health issues, and they were fully informed both verbally and in writing about the study before giving their informed consent. The study was approved by the Institutional Ethics Committee and was carried out in line with the Declaration of Helsinki.

### 4.2.2 Experimental design

All participants were asked to maintain their normal diet and activity throughout the study period and to refrain from alcohol intake and any form of intense physical activity for 24 h prior to each session. Participants performed three baseline measurements on three different occasions, separated by 48h, to determine their  $\dot{V}O_{2\text{peak}}$ , critical power (CP) and 10-km time trial performance, respectively. They were then assigned to either active recovery group (ARG) (N = 7; M: 4; F: 3) or passive recovery group (PRG) (N = 7; M: 5; F: 2) according to their baseline  $\dot{V}O_{2\text{peak}}$ , CP and time-trial performance to ensure that both groups possessed similar baseline values before the training intervention (Table 4.1, 4.2 & Figure 4.1). Furthermore, 7 (M: 4, F: 3) out of 14 participants were randomly allocated to control group (CON) and they repeated the same baseline measurements 2 weeks (males) or 4 weeks (females) apart without performing any training intervention. Female participants of CON performed the physiological and performance measurements 4 weeks apart to ensure that they

completed these measurements at the same stage of their menstrual cycles. Due to the same reason, female participants from both training groups commenced their 2-week training interventions 2 weeks after they had completed the baseline measurements. 3 out of 5 female participants were taking oral contraceptive pills (OCP) during the study period but dose and type remained constant throughout. All participants performed each session at a similar time of day ( $\pm 2$ h) in a controlled environment throughout the study period.

#### *4.2.3 Peak oxygen uptake and other performance measurements during incremental test*

On the initial visit, participants reported to the Human Performance Laboratory after a 4-h fast prior to an incremental test. Body composition was recorded on a calibrated bioelectrical impedance meter (SC-330ST Tanita Body Composition Analyser, Tanita Europe BV, Amsterdam, Netherlands) where body fat and mass were recorded (Table 4.1). The participants then performed an incremental test to determine their  $\dot{V}O_{2\text{peak}}$ , HR<sub>max</sub>, P<sub>max</sub>, time to exhaustion (described in the section 2.2.3.2) and crossover point (section 2.2.8).

#### *4.2.4 Cardiorespiratory measurement during critical power test*

On the second visit, they performed a 3-min all-out cycling test to determine their critical power, 3-min total work, and power produced over each 30-s block (section 2.2.9). Prior to starting the test, participants were connected to a breath by breath oxygen analyser (Metalyzer®3B gas analyser, Cortex, Leipzig, Germany) and had a dampened heart rate monitor attached (Polar Electro, Kempe, Finland) to record  $\dot{V}O_2$ ,  $\dot{V}CO_2$  and heart rate during the test. Cardiorespiratory measures were averaged every 30 sec and the highest and average  $\dot{V}O_2$ ,  $\dot{V}CO_2$  and HR over 3 minutes were determined. Further,  $\dot{V}O_2$ ,  $\dot{V}CO_2$  and HR during each 30-s block over the last 2 min of the test were also analysed in an attempt to assess changes in aerobic metabolism. The first minute was discarded for this particular analysis

since ATP production is mainly supported by anaerobic metabolism during the initial phases of maximal exercise (Gastin 2001). Cardiorespiratory data for ARG only include 6 participants due to a mechanical error with the oxygen analyser occurred during the post-test in one participant.

#### *4.2.5 Cardiorespiratory measurement during 10-km cycling time trial*

On the third visit, the participants performed 10-km time trial as described in the section 2.2.10. Prior to starting the test, participants were connected to a breath by breath oxygen analyser (Metalyzer®3B gas analyser, Cortex, Leipzig, Germany) and had a dampened heart rate monitor attached (Polar Electro, Kempe, Finland) to record  $\dot{V}O_2$ ,  $\dot{V}CO_2$  and heart rate during the test.  $\dot{V}O_2$ ,  $\dot{V}CO_2$  and heart rate were averaged every 30 sec and these variables were normalised to percentage of total time using a cubic spline method (Figure 4.2).

Cardiorespiratory data for ARG only include 6 participants due to a mechanical error with the oxygen analyser occurred during the post-test in one participant (the same participant in the critical power test).

#### *4.2.6 Training sessions*

Both training groups completed the identical training protocol consisting of four to six 30-s sprints against 7.5% bodyweight interspersed with 4-min recovery. However, only ARG cycled at 40%  $\dot{V}O_{2peak}$  during the recovery while PRG remained stationary on the bike as described in the section 2.2.4.3. Both groups performed their respective training protocol three times per week for 2 weeks (6 sessions in total) and sprint load increased with time (4 sprints for the first 2 sessions, 5 sprints for the mid two sessions and 6 sprints for the last two sessions) as previously reported (Gibala et al. 2006). Repeated sprint performance (peak and average power), cardiorespiratory responses ( $\dot{V}O_2$ ,  $\dot{V}CO_2$  and HR) and blood lactate

accumulation over the first 4 sprints in the first and last training sessions were determined, details of which are described in the sections 2.2.4.3, 2.2.5.3 and 2.2.6.2, respectively.

Cardiorespiratory data for PRG only include 6 participants due to increased feelings of discomfort and nausea in one participant resulting from wearing the measuring equipment.

#### *4.2.7 Post intervention tests*

A minimum of 48 hours and maximum of 72 hours after the last training sessions, the participants performed the post-intervention tests. The order of the measurements was identical to the pre-intervention tests.

#### *4.2.8 Statistical analyses*

All data are presented as means  $\pm$  SD. Before conducting parametric tests, the Shapiro-Wilk test was performed to ensure that all values were normally distributed. Effects of training on each variable were analysed using a two-way analysis of variance (ANOVA) with between (group) and repeated (time) factors. Where the analyses revealed a significant main or interaction effect, individual paired samples t-tests were performed to determine the origin of such effects. The normality for a sample distribution derived from the difference between paired scores was determined by the Shapiro-Wilk test, and all sampling distributions were assumed to be normal. Where appropriate, Cohen's  $d$  was calculated to quantify the magnitude of difference within or between subjects. In the case of a within-subjects factor, it was corrected for dependence between means using the equation suggested by Morris and DeShon (2002);  $d = M_{\text{diff}} / SD_{\text{pooled}} \sqrt{2(1 - r)}$ , where  $M_{\text{diff}}$  is mean difference between conditions,  $SD_{\text{pooled}}$  is pooled standard deviation, and  $r$  is correlation between means. Cohen's effect size was defined as follows:  $d < 0.2$  trivial effect, 0.2 - 0.5 small effect, 0.6 -

1.1 moderate effect and 1.2 – 1.9 as a large effect (Cohen 1992). All statistics were run on IBM® SPSS® Version 22.0 for Windows and the level of significance was set at  $P < 0.05$ .

## 4.3 Results

### 4.3.1 Resting measures

There was no change in resting measures following 2 weeks of HIT or in the control group (Table 4.1).

**Table 4.1** Resting measures before and after the experimental period

	ARG (n = 7)		PRG (n = 7)		CON (n = 7)	
	Pre	Post	Pre	Post	Pre	Post
Age (year)	23 ± 3	-	25 ± 4	-	26 ± 4	-
Height (cm)	173.0 ± 11.7	-	172.2 ± 10.7	-	172.0 ± 11.9	-
Body mass (kg)	72.7 ± 16.1	72.9 ± 16.3	71.1 ± 12.9	72.3 ± 13.4	70.2 ± 17.4	70.0 ± 17.0
Fat (%)	20.6 ± 8.6	19.8 ± 8.6	16.7 ± 11.8	16.4 ± 12.2	20.1 ± 9.2	20.0 ± 9.5
SBP (mm Hg)	117 ± 13	124 ± 14	126 ± 15	122 ± 12	122 ± 10	118 ± 13
DBP (mm Hg)	74 ± 9	75 ± 12	79 ± 22	70 ± 10	76 ± 8	73 ± 8
PP (mm Hg)	43 ± 9	49 ± 17	51 ± 8	55 ± 12	46 ± 12	45 ± 8
MAP (mm Hg)	90 ± 13	88 ± 12	91 ± 16	87 ± 14	91 ± 10	86 ± 12
HR rest (beats·min <sup>-1</sup> )	77 ± 13	75 ± 14	73 ± 9	73 ± 16	72 ± 9	76 ± 13

Values are means ± SD. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure. No time by group interaction effect or main difference between groups was observed in all resting measures. A 2-way analysis of variance with between (group) and repeated (time) factors showed no main effect for time, time by group interaction effect or main difference between groups in all resting measures.



#### 4.3.2 Performance measures in the incremental tests

All performance measures during the incremental test were similar in all groups at baseline (Table 4.2). The control group was unchanged after 2 weeks for all performance measures (Table 4.2) and  $\dot{V}O_{2\text{peak}}$  and HR max were unchanged following 2 weeks of HIT (Table 4.2). However, time to exhaustion was significantly increased pre to post in the active recovery group only (6.3%,  $d = 0.98$ ,  $P < 0.05$ , Table 4.2). There was also a tendency for ARG to increase Pmax (5.2%,  $d = 0.88$ ,  $P = 0.06$ ) following 2 weeks of HIT (Table 4.2).

**Table 4.2** Performance measures during the incremental test before and after the experimental period

	ARG (n = 7)		PRG (n = 7)		CON (n = 7)	
	Pre	Post	Pre	Post	Pre	Post
$\dot{V}O_{2\text{peak}}$ (ml <sup>-1</sup> ·kg <sup>-1</sup> ·min <sup>-1</sup> )	37.1 ± 6.0	37.3 ± 7.4	36.8 ± 7.2	36.4 ± 6.9	34.8 ± 6.9	36.8 ± 5.6
$\dot{V}O_{2\text{peak}}$ (l <sup>-1</sup> ·min <sup>-1</sup> )	2.70 ± 0.69	2.75 ± 0.87	2.61 ± 0.67	2.60 ± 0.55	2.47 ± 0.85	2.59 ± 0.69
HR max (beats·min <sup>-1</sup> )	182 ± 5	180 ± 9	180 ± 10	177 ± 10	184 ± 5	181 ± 8
Pmax (watts)*	210 ± 45	221 ± 47	210 ± 56	212 ± 55	203 ± 48	208 ± 54
TTE (sec)*	1021 ± 230	1085 ± 242 <sup>‡</sup>	1021 ± 287	1029 ± 286	982 ± 248	1012 ± 276
Crossover point (watts)	55 ± 15	51 ± 14	56 ± 10	57 ± 11	60 ± 17	56 ± 13
Percentage of Pmax at COP (%)	28 ± 11	25 ± 9	27 ± 4	28 ± 6	30 ± 5	28 ± 7

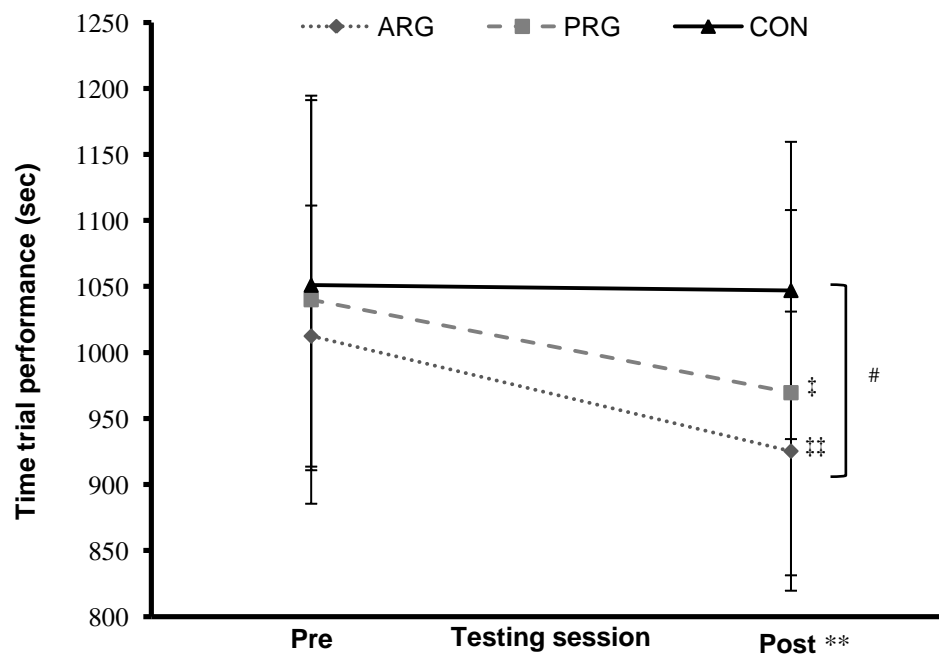
Values are means ± SD.  $\dot{V}O_{2\text{peak}}$ , peak oxygen uptake; HR max, maximal heart rate; Pmax, maximal power output; TTE, time to exhaustion; COP, crossover point. \*Indicates main effect for time ( $p < 0.05$ ). <sup>‡</sup>Indicates  $P < 0.05$  vs. pre within the same group. Main effect for time was detected in a 2-way mixed ANOVA, whereas paired t tests were performed to determine pre to post differences within the same group.

#### 4.3.3 10-km time trial performance

10-km time trial performance was similar in all groups at baseline (ARG: 1012 ± 99 sec, PRG: 1040 ± 155 sec, CON: 1051 ± 140 sec, Fig. 4.1). The control group was unchanged

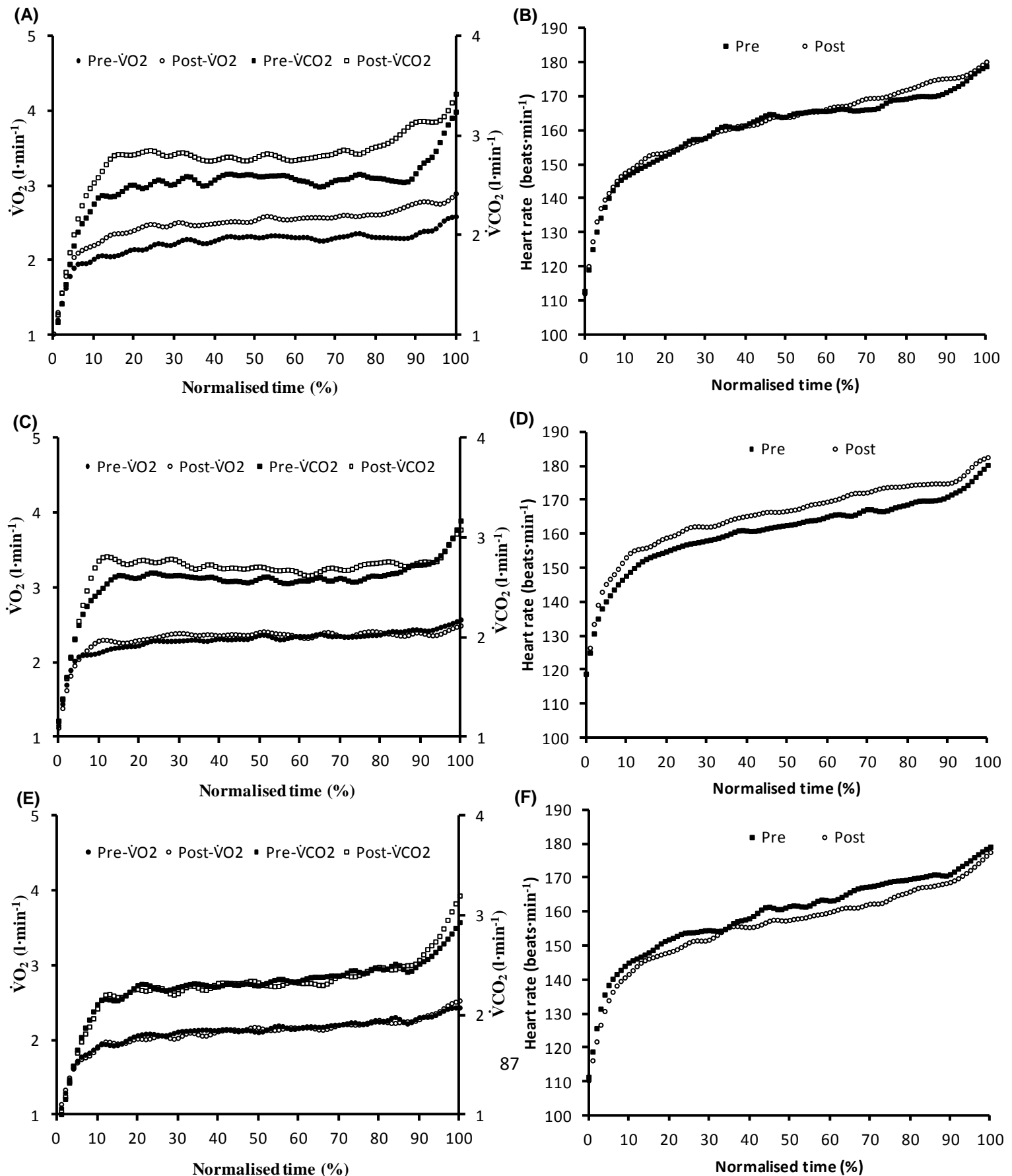
after 2 weeks (pre vs. post:  $1051 \pm 140$  vs.  $1047 \pm 113$  sec,  $P = 0.84$ ); however, 10-km time trial performance was significantly improved pre to post in both ARG and PRG (ARG:  $1012 \pm 99$  to  $925 \pm 106$  sec,  $P < 0.01$ ,  $d = 1.60$ ; PRG:  $1040 \pm 155$  to  $970 \pm 138$  sec,  $P < 0.05$ ,  $d = 0.96$ , Fig. 4.1). There was a trend for  $\dot{V}O_2$  and  $\dot{V}CO_2$  during the time trial to be increased from pre to post in ARG ( $\dot{V}O_2$ :  $2.22 \pm 0.53$  to  $2.48 \pm 0.68$  l·min<sup>-1</sup>,  $P = 0.068$ ;  $\dot{V}CO_2$ :  $2.50 \pm 0.66$  to  $2.76 \pm 0.83$  l·min<sup>-1</sup>,  $P = 0.091$ , Fig. 4.2A), whereas heart rate remained unchanged in all groups (Fig. 4.2B, 4.2D & 4.2F).

**Figure 4.1** Time taken to complete self-paced 10-km time trials before and after the experimental period



\*\*Indicates main effect for time ( $P < 0.01$ ). #Indicates time by group interaction effect ( $P < 0.05$ ). ‡Indicates  $P < 0.01$  vs. pre within the same group. †Indicates  $P < 0.05$  vs. pre within the same group. Main effect of time and time by group interaction were detected in a 2-way mixed ANOVA, whereas paired t tests were performed to determine pre to post differences within the same group.

**Figure 4.2**  $\dot{V}O_2$ ,  $\dot{V}CO_2$  and HR during the 10-km time trials in active recovery group (A & B), passive recovery group (C & D) and control group (E & F). Error bars are not shown for clarity. Cardiorespiratory data for ARG only include 6 participants.



#### 4.3.4 3-min all out cycling tests

Critical power was similar in all groups at baseline (Table 4.3) and remained unchanged in PRG and CON after 2 weeks (Table 4.3). Following 2 weeks of HIT with active recovery, critical power was significantly increased by 7.9% ( $P < 0.05$ ,  $d = 1.75$ , Table 4.2). PRG and CON were also unchanged after 2 weeks for 3-min total work (Table 4.3) and power production over each 30-s block throughout 3 minutes (Fig. 4.3B & 4.3C). In ARG, the total work was significantly increased by 3.7% following 2 weeks ( $P < 0.05$ ,  $d = 1.84$ , Table 4.3), and there was a trend for 30-s power production to be increased with the elapsed time pre to post, reaching a significance during the 5<sup>th</sup> 30-s ( $6.0 \pm 1.5$  to  $6.4 \pm 1.8$  kJ,  $P < 0.01$ ,  $d = 2.53$ ) and 6<sup>th</sup> 30-s blocks ( $6.0 \pm 1.5$  to  $6.4 \pm 1.7$  kJ,  $P < 0.05$ ,  $d = 1.45$ ) (Fig. 4.3A).

During the critical power test,  $\dot{V}O_{2peak}$ , average  $\dot{V}O_2$  and  $\dot{V}CO_{2peak}$  remained unchanged in all groups, while average  $\dot{V}CO_2$  was significantly increased in CON and peak and average heart rates were significantly decreased in ARG after 2 weeks (Table 4.2).  $\dot{V}O_2$  and  $\dot{V}CO_2$  remained unchanged during the last 2 min of the test in all groups (Fig. 4.4A, 4.4C & 4.4E), while heart rate was significantly decreased throughout after 2 weeks in ARG (Fig. 4.4B).

**Table 4.3** Performance parameters and cardiorespiratory responses during the 3-min all-out cycling tests

	ARG		PRG		CON	
	Pre	Post	Pre	Post	Pre	Post
Critical Power (W)**#	202 ± 50	218 ± 59 <sup>‡</sup>	192 ± 46	193 ± 49	188 ± 53	192 ± 49
Total work (kJ)*	46.5 ± 12.1	48.2 ± 13.3 <sup>‡</sup>	44.5 ± 9.5	44.9 ± 10.2	44.2 ± 12.1	44.6 ± 12.5
$\dot{V}O_{2\text{ peak}}$ (l·min <sup>-1</sup> )	2.66 ± 0.77	2.62 ± 0.85	2.67 ± 0.61	2.69 ± 0.63	2.36 ± 0.78	2.52 ± 0.70
$\dot{V}O_{2\text{ average}}$ (l·min <sup>-1</sup> )	2.34 ± 0.76	2.36 ± 0.75	2.42 ± 0.54	2.40 ± 0.56	2.10 ± 0.67	2.22 ± 0.65
$\dot{V}CO_{2\text{ peak}}$ (l·min <sup>-1</sup> )	4.17 ± 1.63	4.44 ± 1.53	4.26 ± 0.99	4.29 ± 0.96	3.89 ± 1.45	4.04 ± 1.39
$\dot{V}CO_{2\text{ average}}$ (l·min <sup>-1</sup> )*	3.46 ± 1.33	3.77 ± 1.26	3.66 ± 0.87	3.68 ± 0.84	3.23 ± 1.16	3.41 ± 1.19 <sup>‡‡</sup>
HR <sub>peak</sub> (beats·min <sup>-1</sup> )**	175 ± 7	171 ± 7 <sup>‡‡</sup>	176 ± 10	173 ± 10	176 ± 8	174 ± 7
HR <sub>average</sub> (beats·min <sup>-1</sup> )*	167 ± 8	161 ± 8 <sup>‡‡</sup>	167 ± 10	166 ± 11	168 ± 8	166 ± 7

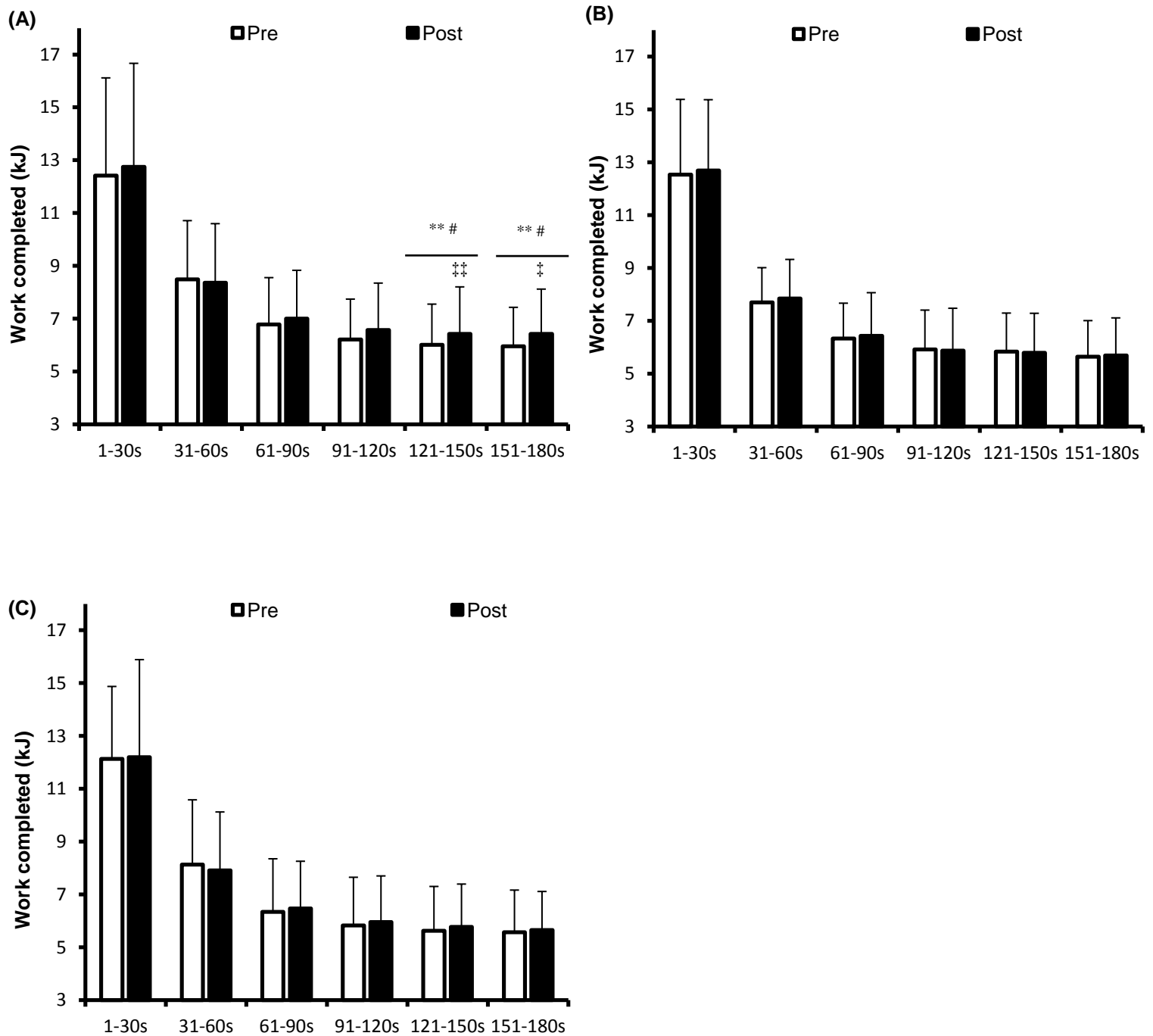
Values are means ± SD. \*\*Indicates main effect for time ( $P < 0.01$ ). \*Indicates main effect for time ( $P < 0.05$ ).

#Indicates time by group interaction effect ( $P < 0.05$ ). <sup>‡‡</sup>Indicates  $P < 0.01$  vs. pre within the same group.

<sup>‡</sup>Indicates  $P < 0.05$  vs. pre within the same group. Cardiorespiratory data for ARG only include 6 participants.

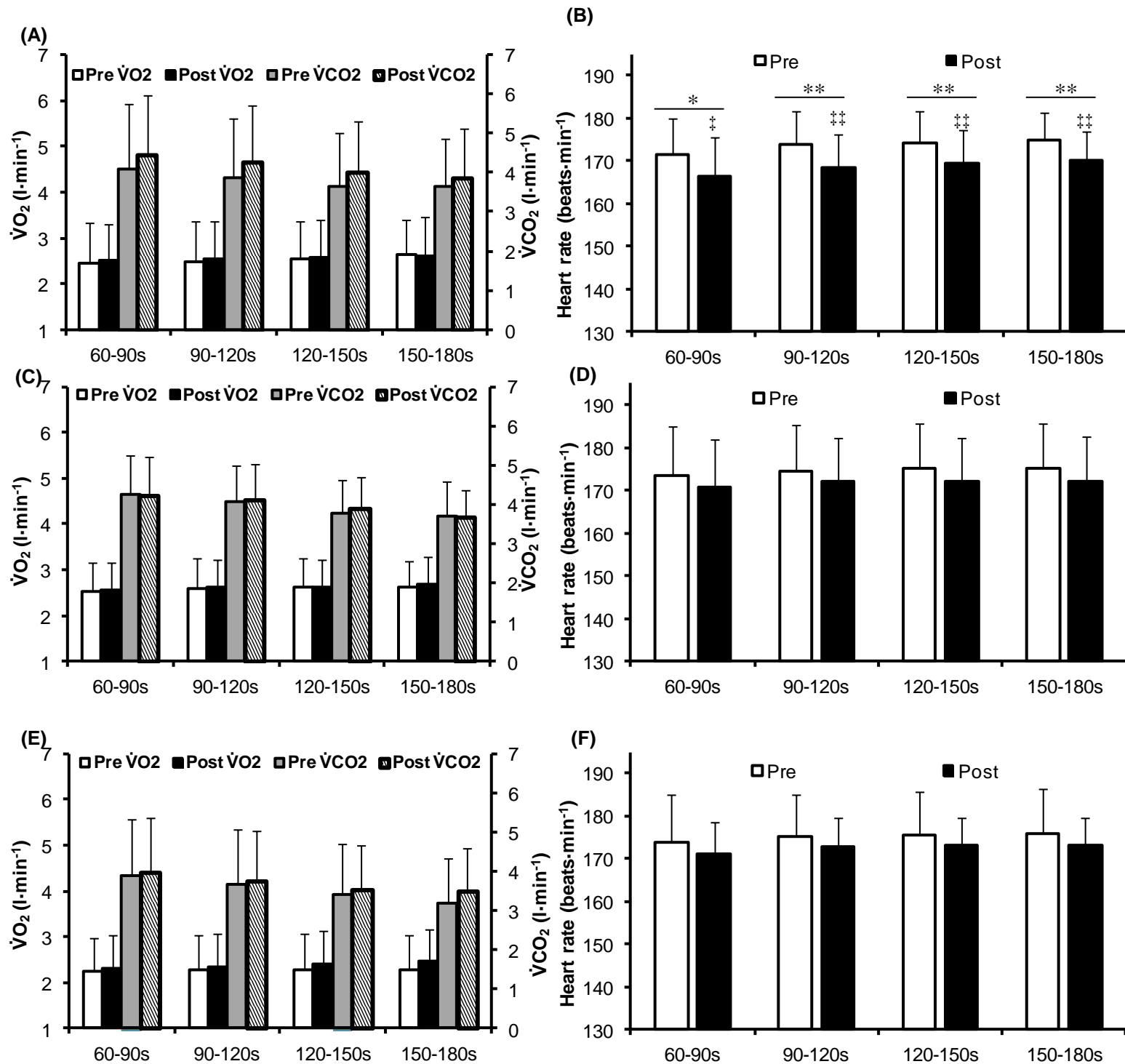
Statistical analyses were performed as described in the legends of the tables and figures above.

**Figure 4.3** Power produced over each 30-sec section during the 3-min all-out tests in active recovery group (A), passive recovery group (B) and control group (C)



\*\*Indicates main effect for time ( $P < 0.01$ ). #Indicates time by group interaction effect ( $P < 0.05$ ). \*\*Indicates  $P < 0.01$  vs. pre within the same group. †Indicates  $P < 0.05$  vs. pre within the same group. Statistical analyses were performed as described in the legends of the tables and figures above.

**Figure 4.4**  $\dot{V}O_2$ ,  $\dot{V}CO_2$  and HR during the last 2 minutes of the 3-min all-out tests in active recovery group (A & B), passive recovery group (C & D) and control group (E & F)



\*\*Indicates main effect for time ( $P < 0.01$ ). \*Indicates main effect for time ( $P < 0.05$ ). \*\*Indicates  $P < 0.01$  vs. pre within the same group. \*Indicates  $P < 0.05$  vs. pre within the same group. Cardiorespiratory data for ARG only include 6 participants. Statistical analyses were performed as described in the legends of the tables and figures above.

#### *4.3.5 Wingate performance and cardiorespiratory and blood lactate responses during the training sessions*

Wingate performance parameters (peak and average power over the 4 sprints) and the level of blood lactate accumulation at baseline were similar between the training groups and those parameters remained unchanged from session 1 to 6 in both ARG and PRG (Table 4.4). There was no difference between the groups in  $\dot{V}O_2$ ,  $\dot{V}CO_2$  and HR during the sprints, whereas all cardiorespiratory variables were significantly elevated in ARG compared to PRG during the recovery intervals (recovery  $\dot{V}O_2$ : ARG > PRG,  $P < 0.01$ ,  $d = 2.25$ ; recovery  $\dot{V}CO_2$ : ARG > PRG,  $P < 0.01$ ,  $d = 1.51$ ; recovery HR: ARG > PRG,  $P < 0.05$ ,  $d = 1.58$ , Table 4.4). Whilst recovery  $\dot{V}O_2$  was significantly increased in ARG from session 1 to 6 ( $P < 0.05$ ,  $d = 1.02$ , Table 4.4), other cardiorespiratory measures were not significantly altered from session 1 to 6 in either group (Table 4.4). Examples of cardiorespiratory responses during the sprint and recovery periods in each training group are presented in Figure 4.5.

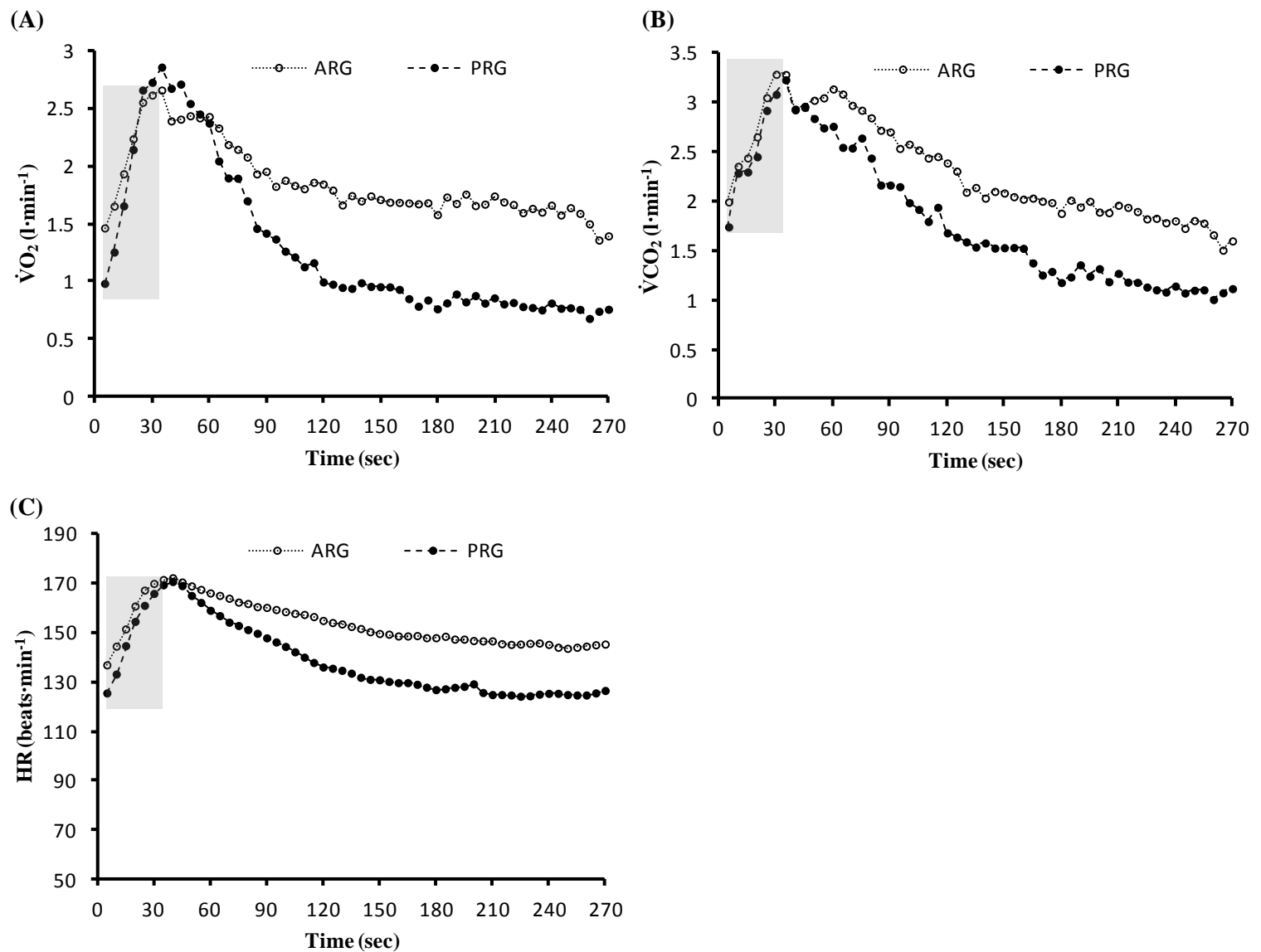


**Table 4.4** Wingate performance, cardiorespiratory and blood lactate responses during the first and last training sessions

	ARG		PRG	
	Session 1	Session 6	Session 1	Session 6
$\dot{V}O_{2 \text{ average}}$ over 4 sprints ( $\text{l}\cdot\text{min}^{-1}$ )	$1.94 \pm 0.61$	$2.02 \pm 0.61$	$1.80 \pm 0.36$	$1.76 \pm 0.25$
$\dot{V}O_{2 \text{ average}}$ over 4 rest periods( $\text{l}\cdot\text{min}^{-1}$ )* <sup>§§</sup>	$1.77 \pm 0.40$	$1.85 \pm 0.42^{\ddagger}$	$1.16 \pm 0.16$	$1.21 \pm 0.15$
$\dot{V}CO_{2 \text{ average}}$ over 4 sprints ( $\text{l}\cdot\text{min}^{-1}$ )	$2.38 \pm 0.84$	$2.43 \pm 0.70$	$2.21 \pm 0.60$	$2.07 \pm 0.25$
$\dot{V}CO_{2 \text{ average}}$ over 4 rest periods ( $\text{l}\cdot\text{min}^{-1}$ ) <sup>§</sup>	$2.23 \pm 0.66$	$2.36 \pm 0.62$	$1.64 \pm 0.22$	$1.69 \pm 0.20$
HR <sub>average</sub> over 4 sprints ( $\text{beats}\cdot\text{min}^{-1}$ )	$154 \pm 10$	$152 \pm 10$	$147 \pm 11$	$143 \pm 11$
HR <sub>average</sub> over 4 rest periods ( $\text{beats}\cdot\text{min}^{-1}$ ) <sup>§</sup>	$152 \pm 11$	$153 \pm 11$	$136 \pm 15$	$132 \pm 12$
Peak power over 4 sprints ( $\text{W}\cdot\text{kg}^{-1}$ )	$10.1 \pm 2.3$	$10.3 \pm 2.3$	$9.4 \pm 1.6$	$9.9 \pm 1.8$
Average power over 4 sprints ( $\text{W}\cdot\text{kg}^{-1}$ )	$6.7 \pm 1.0$	$6.9 \pm 0.9$	$6.6 \pm 0.9$	$6.8 \pm 0.9$
Peak blood lactate ( $\text{mmol}\cdot\text{l}^{-1}$ )	$14.4 \pm 1.8$	$14.8 \pm 1.0$	$14.0 \pm 1.6$	$14.8 \pm 0.7$
Average blood lactate ( $\text{mmol}\cdot\text{l}^{-1}$ )	$12.2 \pm 1.6$	$13.1 \pm 0.8$	$12.9 \pm 1.6$	$13.4 \pm 0.7$

Values are means  $\pm$  SD. \*Indicates main effect for time ( $P < 0.05$ ). <sup>§§</sup>Indicates significant difference between groups ( $P < 0.01$ ). <sup>§</sup>Indicates significant difference between groups ( $P < 0.05$ ). <sup>‡</sup>Indicates  $P < 0.05$  vs. pre within the same group. Cardiorespiratory data for PRG only include 6 participants. Statistical analyses were performed as described in the legends of the tables and figures above.

**Figure 4.5** Examples of cardiorespiratory responses during training (group mean): oxygen uptake (A), carbon dioxide production (B) and heart rate (C)



Error bars are not shown for clarity. Shaded areas indicate the sprint period. Cardiorespiratory data for PRG only include 6 participants.

#### 4.4 Discussion

The present study demonstrated that endurance performance adaptations are dependent upon recovery mode during typical Wingate-based high-intensity training over 2 weeks (4 to 6 30-s sprints interspersed with 4-min recovery against 7.5% bodyweight). Although both training

groups improved time-trial performance, only the active recovery group increased critical power, 3-min total work and time to exhaustion. The greater performance adaptations seen in ARG after the training (Table 4.2 & 4.3) seem to reflect improved O<sub>2</sub> consumption and utilisation, suggesting that the elevated aerobic metabolism achieved by ARG during the training (Table 4.4) may have allowed greater muscle oxidative adaptations compared with the passive recovery group.

#### *4.4.1 Resting cardiovascular measurement*

In line with the previous 2-week Wingate-based HIT study (Astorino et al. 2012), we did not see any changes in resting HR or blood pressure. In contrast, Whyte et al. (2010) saw a significant reduction in systolic BP following 2 weeks of Wingate-based HIT in sedentary overweight/obese men ( $32.1 \pm 8.7$  years; BMI:  $31.0 \pm 3.7 \text{ kg}\cdot\text{m}^{-2}$ ). This indicates that whilst sprint interval training may provide an improvement in resting blood pressure in less active, overweight/obese populations, such effect may not be confirmed in healthy normotensive participants in response to HIT (Whyte et al. 2010; Astorino et al. 2012).

#### *4.4.2 Performance measures during the incremental test*

There was no improvement in  $\dot{V}\text{O}_{2\text{peak}}$  in either training group in the present study (Table 4.2). This is similar to what has been shown previously when using the same training protocol (Burgomaster et al. 2005, 2006). When longer recovery, greater resistive force or more sprints are employed, then 2 weeks of Wingate protocols have been shown to improve  $\dot{V}\text{O}_{2\text{max}}$  (Bailey et al. 2009; Hazell et al. 2010; Whyte et al. 2010; Astorino et al. 2012). Exercise intensity (i.e. the level of power production) has been suggested to be a key factor in inducing oxidative adaptations in skeletal muscles, type II fibres in particular (Bailey et al. 2009; Buchheit et al. 2012; Sloth et al. 2013), and therefore the selection of recovery duration

and/or resistive load may have an impact on overall aerobic adaptations (Baker et al. 2009; Hazell et al. 2010; Zelt et al. 2014). Although Bailey et al. (2009) employed the traditional resistive load (7.5% TBM) as well as recovery duration (4 min), greater number of sprints were performed (35 sprints in total) in their study compared to the aforementioned 2-week Wingate studies or the current study (30 sprints in total). Given that muscle  $O_2$  extraction has been shown to be increased with successive sprints (Buchheit et al. 2012), the greater sprint repetitions in their study might have allowed an increased  $O_2$  utilisation during the training, and consequently improved  $\dot{V}O_{2peak}$ .

Following 2 weeks of HIT, improvements in time to exhaustion were only seen in ARG. During the training, the active recovery resulted in greater aerobic metabolic demands following sprints (Table 4.4 & Figure 4.5), which might have resulted in greater peripheral adaptations. Previously, increases in time to exhaustion were associated with improvements in mitochondrial function following 2 weeks of Wingate-based HIT (Burgomaster et al. 2005) or 8 weeks of high-intensity aerobic interval training at  $\sim 90\% \dot{V}O_{2max}$  (Daussin et al. 2008). Daussin et al. (2008) also associated the increased TTE with faster  $\dot{V}O_2$  kinetics resulting from an enhanced muscle  $O_2$  uptake. Likewise, Bailey et al. (2009) showed accelerated  $\dot{V}O_2$  kinetics and muscle  $O_2$  extraction during moderate- and severe-intensity exercise following 2 weeks of Wingate-based HIT. Accelerated  $\dot{V}O_2$  kinetics and muscle  $O_2$  uptake may delay the onset of depletion of muscle high-energy phosphates (e.g. PCr) and accumulation of fatigue related metabolites (e.g.  $H^+$ ,  $P_i$ ) (Jones et al. 2008; Vanhatalo et al. 2010), which likely results in an enhanced exercise tolerance (Bailey et al. 2009). Moreover, Daussin et al. (2008) also demonstrated an increased TTE in their continuous training group (20 to 35 min cycling at  $\sim 61\% \dot{V}O_{2max}$ ) in the absence of mitochondrial adaptation but due to greater improvements in capillary density and vascular conductance compared to the high-intensity training group.

This indicates that improved muscle perfusion and thus O<sub>2</sub> supply in addition to enhanced muscle O<sub>2</sub> uptake may increase tolerable exercise duration (Daussin et al. 2008). Following 4 weeks of high-intensity training consisting of repeated one-legged knee extensor exercise at 150% of leg  $\dot{V}O_{2\max}$ , there was an increased capillary density in both type I and II muscle fibres (Jensen et al. 2004). Therefore, it is possible that improvements in capillary density following HIT as well as mitochondrial adaptations are regulating the improvement in time to exhaustion. Given that Gibala et al. (2006) showed increased muscle buffering capacity following 2 weeks of Wingate-based HIT, an improved buffering capacity could be another candidate for the increased TTE in ARG. However, a similar level of blood lactate accumulation was observed during training between the groups (Table 4.4), which may indicate that both sprint training protocols provided a similar magnitude of stimulus for adaptations of the muscle pH regulation systems (Bishop et al. 2011). Furthermore, neither training group improved repeated Wingate performance following 2 weeks of HIT (Table 4.4) and considering that an increased muscle buffer capacity has been associated with an improved repeated sprint ability (Ross and Leveritt 2001; Bishop et al. 2004; Edge et al. 2006), it is unlikely that there was a significant increase in buffering capacity in either training group. Therefore, the increased time to exhaustion achieved by ARG does not seem to be attributed to an increased muscle buffer capacity in the present study.

Crossover point represents the work rate at which CHO-derived fuels become predominant over those derived from lipids for energy production (Brooks and Mercier 1994; Brooks 1997). Therefore, an increase in COP would suggest an increased fat oxidation rate or altered substrate utilisation during exercise. Neither crossover point nor relative intensity at COP was altered following 2 weeks of HIT irrespective of recovery mode (Table 4.2). This is partially in line with the previous Wingate-based studies which did not see changes in mitochondrial

makers for skeletal muscle lipid oxidation (3-hydroxyacyl CoA dehydrogenase; Burgomaster et al. 2006, 2007). Higher training volume may be required to increase lipid oxidation with HIT (Gibala and McGee 2008) either via longer training intervention (> 6 weeks) (Burgomaster et al. 2008) or longer work intervals (e.g. aerobic-type interval training) (Talanian et al. 2007).

#### *4.4.3 Time trial performance*

Both groups improved the 10-km time trial performance after the training (ARG: 8.6%; PRG: 6.7%; Fig. 4.1) to a similar extent. The magnitude of improvement is comparable to the previous 2-week Wingate-based studies that report improvements of between 5 to 10% in 5-km (Hazell et al. 2010), 750-kJ (Gibala et al. 2006) and 250-kJ (Burgomaster et al. 2006; Babraj et al. 2009) cycling time trials. The improvement in time trial performance occurs without any improvement in  $\dot{V}O_{2peak}$  but with increased activity of mitochondrial enzymes such as citrate synthase and pyruvate dehydrogenase (Burgomaster et al. 2006). Therefore, the observed time-trial improvements in the present study may reflect an improved muscle oxidative potential previously seen after 2 weeks of Wingate-based exercise training (Burgomaster et al. 2005, 2006; Gibala et al. 2006). The improved performance seen after both training interventions (ARG vs. PRG) in the current study suggests that fluctuations of workload and  $\dot{V}O_2$  rather than overall aerobic metabolism during the training may be important for increasing muscle oxidative potential and thus aerobic performance (Daussin et al. 2008; Cochran et al. 2014).

Nevertheless, active recovery might bring about a greater peripheral oxidative adaptation compared to passive recovery. Given the lack of improvement in  $\dot{V}O_{2peak}$  in the present study, it would appear that there was limited change in central factors and that  $O_2$  delivery (cardiac

output) to working muscles during any post-performance test should be the same unless heart rate increased (Bassett and Howley 2000; Laursen and Jenkins 2002). In the current study, there was a trend for greater  $\dot{V}O_2$  and  $\dot{V}CO_2$  in ARG, but not PRG or CON, during the post-trial and heart rate was almost identical between the pre- and post-tests for all groups (Figure 4.2 A-F). This may indicate that in ARG, the working muscles consumed more  $O_2$  with similar oxygen availability (Bailey et al. 2009) and produced a greater amount of oxidative ATP as reflected by the increased  $\dot{V}CO_2$  (McKenna et al. 1997a). It also could be argued that there were greater improvements in capillary density in ARG compared to PRG, enabling improvements in muscle perfusion and thus  $O_2$  supply without changes in central mechanisms (Daussin et al. 2008). Moreover, during the time trials,  $\dot{V}CO_2$  was consistently greater than  $\dot{V}O_2$  (i.e. RER > 1) in all groups (Figure 4.2A, C & E), suggesting bicarbonate buffering of lactic acid-derived hydrogen ion (Péronnet and Aguilaniu 2006; Whipp 2007). Therefore, although lactate accumulation was not measured, it seems that there was an increased rate of anaerobic glycolysis in addition to oxidative metabolism in all groups during the time trials. Furthermore, all groups showed rapid increases in  $\dot{V}O_2$  and  $\dot{V}CO_2$  at the end of the pre-and post trials (Figure 4.2 A, C & E), which indicates last moment increases in effort. In the current study, the amount of distance covered was visible on the screen during the tests, and thus pacing was not tightly controlled, possibly leading to the observed phenomenon. Nevertheless, whilst CON did not improve time-trial performance following 2 weeks, both ARG and PRG showed improvements in the performance after 2 weeks of HIT (Figure 4.1), indicating that the improvements seen in the training groups were not attributed to an adoption of pacing strategy or learning effect but adaptations to HIT.

#### 4.4.4 Critical power

Zelt et al. (2014) recently demonstrated that 4 weeks of four to six 15-s or 30-s cycling sprints increased critical power by approximately 5 to 7%, suggesting that sprint-type (all-out) interval training can also improve this parameter in addition to submaximal endurance training (Gaesser and Wilson 1988; Jenkins and Quigley 1992) as well as high-intensity but constant-work interval training (Gaesser and Wilson 1988; Pool et al. 1990; Vanhatalo et al. 2008). Vanhatalo et al. (2008) observed a 7% increase in  $\dot{V}O_{2\text{peak}}$  during a 3-min all-out cycling test in addition to a 10% increase in critical power following 12 sessions of aerobic-type interval training over 4 weeks. In the current study, only the active recovery group improved critical power and 3-min total work without any gains in  $\dot{V}O_{2\text{peak}}$  or average  $\dot{V}O_2$  during the 3-min CP test after the intervention (Table 4.3). Furthermore, the improved total work observed in the active recovery group was mostly attributed to the increased power production during the final 90 sec of the test (Fig. 4.3A). This is partially in line with the above studies (Gaesser and Wilson 1988; Pool et al. 1990; Jenkins and Quigley 1992; Vanhatalo et al. 2008) that also improved CP and/or TW with no change in curvature constant ( $W'$ ), which is not exclusively but mainly derived from anaerobic metabolism (Jones et al. 2010). Whilst oxygen uptake remained unchanged over the last 2 min of the test,  $\dot{V}CO_2$  tended to be increased and HR was significantly decreased during the post-CP test in ARG (Fig. 4.4A & 4.4B). Similar to the time trial, this could suggest increased muscle  $O_2$  extraction and utilisation (McKenna et al. 1997a; Tordi et al. 2003; Bailey et al. 2009) or improved muscle  $O_2$  perfusion due to greater capillarisation of the muscle (Daussin et al. 2008) after the training in this group. The increased power production over the second 90 sec of the test would support these assumptions since ATP generation is increasingly derived from oxidative phosphorylation with time during maximal exercise (Gastin 2001; Laursen 2010). Nevertheless, since this study did not directly measure changes in muscle oxygenation



state or capillary density, it has yet to be determined whether these peripheral factors account for greater endurance adaptations with active recovery. Previously, the degree of muscle O<sub>2</sub> extraction has been established from near-infrared spectroscopy (NIRS)-derived tissue oxygenation index (TOI) during repeated 30-s Wingate tests (Buchheit et al. 2012), while increases in capillary density of muscle were confirmed via muscle biopsy following 4 to 8 weeks of HIT (Jensen et al. 2004; Daussin et al. 2008). Furthermore, systemic vascular conductance during exercise has been also estimated by the ratio between cardiac output and mean arterial pressure obtained from bioimpedance method and an inflatable cuff, respectively (Daussin et al. 2008). Therefore, a future study would be able to explore underlying mechanisms responsible for the differences in training adaptations between the recovery modalities using these methods. Although Zelt et al. (2014) also employed unloaded cycling as a recovery modality during rest periods, greater training volume in their study (12 sessions over 4 weeks) compared to the current study (6 sessions over 2 weeks) may have allowed the increased CP in their study. Therefore, it could be assumed that, with active recovery at low to moderate intensity ( $\sim 40\% \dot{V}O_{2\text{peak}}$ ), it induces rapid endurance adaptations and a greater training volume would be required to induce similar improvements with passive or very light active recovery.

#### *4.4.5 Potential influence of menstrual cycle on the study results*

In the present study, the female participants performed the physiological and performance measurements 4 weeks apart in an attempt to minimise the effects of menstrual cycle on each variable. Menstrual history questionnaires from the female participants revealed that, during the study period, one female participant was amenorrheic due to the use of oral contraceptive pills (Cerazette 75 microgram film-coated tablet), whereas the other female participants were generally having regular menstrual cycles (28 to 38 days in length, or  $32.6 \pm 4.4$  d on

average). However, without measuring basal body temperature or hormonal levels (Lebrun et al. 1995), it was not entirely clear whether the female participants completed each measurement at the same stage of their menstrual cycles. Indeed, two out of the three female participants in the control group increased their  $\dot{V}O_{2peak}$  by 5 to 7  $ml \cdot min^{-1} \cdot kg^{-1}$  from pre to post without performing any training intervention. Nevertheless, changes in  $\dot{V}O_{2peak}$  in CON did not reach statistical significance regardless of these participants ( $P = 0.26$  or  $0.93$  for with or without inclusion of the two participants, respectively). Furthermore, one of the two participants who increased  $\dot{V}O_{2peak}$  was amenorrheic during the study period, and the type and dose of her OCP remained constant throughout (Cerazette 75 microgram film-coated tablet), indicating that other factors such as biological day-to-day variation (Midgley et al. 2006b) other than menstrual cycle might have resulted in the increased  $\dot{V}O_{2peak}$  in this participant. In addition, while none of the female participants increased  $\dot{V}O_{2peak}$  following 2 weeks of HIT, four out of five female participants from both training groups improved their time-trial performance (i.e. the female participants showed a similar trend to the male participants). This suggests that although the changes in hormonal levels (e.g. oestrogen, progesterone) with menstrual cycle may cause fluctuations in physiological variables such as plasma volume and haemoglobin concentration, there is an overriding adaptation to training (Frankovich and Lebrun 2000). Therefore, it seems to be reasonable to assume that there was a minimal effect of menstrual cycle on the overall results in the current study.

#### **4.5 Conclusions**

This study demonstrates for the first time that active recovery during sprint-type (all-out) interval training promotes greater endurance performance adaptations than passive recovery over 2 weeks. This may suggest a greater adaptation of peripheral mechanisms as unchanged  $\dot{V}O_{2peak}$  following 2 weeks in both training groups would suggest limited changes in central

factors (Bassett and Howley 2000). Active recovery promotes a greater aerobic metabolic demand during training which results in ~ 6 to 8% greater adaptations in endurance capacity as shown in the increased critical power and time to exhaustion in this study. Therefore, active recovery should be employed during HIT to gain greater training benefits without increasing total training time commitment. In conclusion, it has been demonstrated, for the first time, that active recovery is more effective when performing sprint interval training to improve endurance performance over a short time frame.

# **Chapter 5**

## **Study 3**

## **Effects of reduced-volume of sprint interval training and the time course of physiological and performance adaptations over 9 weeks**

### **5.1 Introduction**

It has been demonstrated that Wingate-based high-intensity training (HIT) consisting of 4 to 6 30-s sprints interspersed with 4 min of recovery promotes comparable metabolic and physiological adaptations (e.g. an improved mitochondrial function) to those obtained from traditional aerobic exercise training (e.g. 60 to 90 min of continuous cycling at 65%  $\dot{V}O_{2\max}$ ) despite its low training volume (2 to 3 min of all-out efforts) (Gibala et al. 2006; Burgomaster et al. 2008). Nevertheless, the conventional 30-s Wingate-based HIT may not be necessarily time-efficient if warm-up and recovery periods are included, amounting to ~30 minutes (Gibala et al. 2006; Burgomaster et al. 2008). It has been shown that the majority of anaerobic metabolism (i.e. the degradation of phosphocreatine and glycogen) occurs within the first 15 sec during a maximal sprint (Bogdanis et al. 1996a, 1998; Parolin et al. 1999), and that aerobic metabolism increases with successive bouts irrespective of sprint duration (6 to 30 sec) when sprints are separated by 30-s to 4-min recovery (Gaitanos et al. 1993; Bogdanis et al. 1996a, 1998; Hargreaves et al. 1998; Parolin et al. 1999). Therefore, shorter sprint protocol ( $\leq 15$  sec) may induce a similar metabolic demand to that seen in Wingate-based HIT and reducing sprint duration may not result in a diminished training adaptation. Indeed, Hazell et al. (2010) demonstrated that a reduced-volume of sprint interval training consisting of 4 to 6 10-s cycling sprints separated by 2- or 4-min recovery was as effective as typical 30-s Wingate-based HIT in improving  $\dot{V}O_{2\max}$ , 5-km time trial and single 30-s Wingate performance. Similarly, Zelt et al. (2014) recently demonstrated that 4 to 6 15-s sprints interspersed with 4.75 min of recovery improved aerobic capacities such as  $\dot{V}O_{2\text{peak}}$ , critical power and lactate threshold to a similar extent compared to the same numbers of 30-s sprints

separated by 4.5-min recovery. Rapid physiological and metabolic adaptations to sprint-type interval training may be related to a high level of power production which induces an increased level of muscle fibre recruitment (Bailey et al. 2009; Buchheit et al. 2012; Sloth et al. 2013). Hazell et al. (2010) found that training intensity (the reproducibility of power during training) was increased in the 10-s training groups compared to the 30-s group and suggested that the level of power production rather than sprint duration would be important for inducing training benefits. Nevertheless, neither sprint duration nor sprint-to-rest ratio was matched among the groups (10:120s, 10:240s, 30:240s) in their study, and thus the improved reproducibility of power might have been attributed to greater sprint: rest ratio rather than shorter sprint duration itself. Indeed, it has recently been demonstrated that work to rest ratio alters the training adaptations to HIT, with 1:3 work to rest ratio producing more aerobic adaptations and 1:12 work to rest ratio producing more power adaptations (Kavaliuskas et al. 2015). Therefore, further research is required to confirm whether shorter sprint protocol with the same sprint: rest ratio as Wingate-based HIT also brings about an improved power restoration during training and consequently comparable training gains.

Despite the increased utilisation of Wingate-based HIT to promote physiological and performance adaptations (Burgomaster et al. 2005, 2006; Gibala et al. 2006; Bailey et al. 2009; Macpherson et al. 2011; Trilk et al. 2011; Astorino et al. 2012), little is known regarding the time course of those adaptations. Whilst Burgomaster et al. (2008) found an increase in  $\dot{V}O_{2peak}$  following 3 weeks of Wingate-based HIT, no further improvements were confirmed with another 3 weeks of the training. This is similar to the findings obtained from a recent study employing aerobic-type high-intensity interval training where the majority of  $\dot{V}O_{2max}$  gains induced by HIT occurred within 4 weeks during a 12-week training intervention (Astorino et al. 2013). The findings from the study by Burgomaster et al. (2008)

or Astorino et al. (2013) may indicate that the vast majority of cardiorespiratory adaptations brought about by HIT are completed within 3 to 4 weeks. Nevertheless, since Burgomaster et al. (2008) conducted  $\dot{V}O_{2\text{peak}}$  measurement at only two different time points over a relatively short timeframe (6 weeks), more frequent assessments over a longer study period would provide a further insight into the time course of physiological adaptations to sprint-type interval training such as Wingate-based HIT.

In short, the purposes of the current study were twofold, that is, to determine 1) effects of sprint duration (15 sec vs. 30 sec) on physiological and performance adaptations while matching sprint-to-rest ratio between training protocols (sprint: rest ratio of 1:8) and 2) time course of those adaptations over 9 weeks (baseline, weeks 3, 6 and 9). It was hypothesised that the difference in sprint duration would not affect overall training adaptations, and that most of the adaptations would occur during the initial phases.

## **5.2 Methods and Materials**

### *5.2.1 Participants*

27 healthy active adults (male: 20, female: 7) who took part in a minimum of 3-h exercise per week initially participated in this study (Table 5.1). However, 1 male participant of each training group withdrew from the study due to injuries unrelated to the study. Consequently, 25 (18 males and 7 females) participants completed the current study. All were physically active but none of them were participating in regular sport competitions during the period of the study. All participants completed a Physical Activity Readiness Questionnaire (PARQ) to ensure there were no underlying health issues, and they were fully informed both verbally and in writing about the study before giving their informed consent. 2 out of 7 female participants were taking oral contraceptive pills during the study period but dose and type

remained constant throughout. The study was approved by the Institutional Ethics Committee and was carried out in line with the Declaration of Helsinki.

### *5.2.2 Experimental design*

All participants were asked to maintain their normal diet and activity throughout the study period and to refrain from alcohol intake and any form of intense physical activity for 24 h prior to each session. Participants performed three baseline measurements on three different occasions, separated by 48h, to determine their peak oxygen uptake ( $\dot{V}O_{2\text{peak}}$ ), critical power (CP) and 10-km cycling time-trial performance respectively. They were then assigned to either 15-s training group consisting of 4 to 6 15-s cycle sprints interspersed with 2-min active recovery at 40% of  $\dot{V}O_{2\text{peak}}$  (15TG) (N = 9; male: 7, female: 2) or 30-s training group consisting of 4 to 6 30-s cycle sprints interspersed with 4-min active recovery at 40% of  $\dot{V}O_{2\text{peak}}$  (30TG) (N = 8; male: 5; female: 3) according to their baseline  $\dot{V}O_{2\text{peak}}$ , CP and time-trial performance to ensure that both groups possessed similar baseline values before the training intervention. Additional eight recreationally active adults (male: 6, female: 2) performed the three baseline measurements 9 weeks apart without performing any training intervention to act as a control group (CON) (Table 5.1). All participants performed each session at a similar time of day ( $\pm 2$ h) in a controlled environment throughout the study period and each session was separated by at least a period of 48h.

### *5.2.3 Peak oxygen uptake and other performance measurements during incremental test*

On the initial visit, participants reported to the laboratory at a time suitable for them after a 4-h fast. Firstly, body composition was recorded. They removed their shoes and socks and had their height measured prior to stepping onto a calibrated bioelectrical impedance meter (SC-330ST Tanita Body Composition Analyser, Tanita Europe BV, Amsterdam, Netherlands)



where body fat mass and lean body mass were recorded (Table 5.1). Resting blood pressure and heart rate were then recorded using an automatic blood pressure monitor (Watch BP<sup>®</sup> office, Microlife Health Management Ltd., Cambridge, UK) after the participants had been seated for 5 minutes. They then performed an incremental test to determine their  $\dot{V}O_{2peak}$ , HRmax, Pmax, time to exhaustion (section 2.2.3.2), peak oxygen pulse (section 2.2.11) and crossover point (section 2.2.8).

#### *5.2.4 3-min all-out cycling test*

On the second visit, they performed a 3-min all-out cycling test to determine their critical power as described in the section 2.2.9.

#### *5.2.5 10-km cycling time trial*

On the third visit, the participants performed a self-paced 10-km cycling time trial as described in the section 2.2.10.

#### *5.2.6 Training intervention*

The training groups performed their respective training protocol against a predetermined resistance (male: 7.5% of bodyweight; female: 6.5% of bodyweight) twice per week over 9 weeks (18 sessions in total) and sprint load increased with time (i.e. 4 sets for the initial 3 weeks, 5 sets for the second 3 weeks and 6 sets for the last 3 weeks). The details of the assessments of repeated sprint performance (section 2.2.4.4), heart rate responses (section 2.2.5.4) and blood lactate (section 2.2.6.3) are already mentioned.

### 5.2.7 Post intervention tests

A minimum of 48 hours and maximum of 72 hours after the last training sessions, participants from the training groups performed the post-intervention tests. The order of the testing was identical to the pre-intervention tests.

### 5.2.8 Statistical analysis

All data are presented as means  $\pm$  SD. Before conducting parametric tests, the Shapiro-Wilk test was performed to ensure that all values were normally distributed. Effects of training on each variable were analysed using a two-way analysis of variance (ANOVA) with between (group) and repeated (pre to post) factors for all groups. A two-way ANOVA was also run with repeated measures (0, 3, 6 and 9 weeks) to determine the time course of physiological and performance adaptations in response to training, with training group used as a between-subjects factor (15TG vs. 30TG). Likewise, heart rate and blood lactate accumulation during training were analysed using a two-way mixed ANOVA. Where the analyses revealed a significant main or interaction effect for all groups, individual paired samples t-tests were performed to determine the origin of such effects. The normality for a sample distribution derived from the difference between paired scores was determined by the Shapiro-Wilk test, and when the normality assumption was violated, Wilcoxon signed-rank test was used. In the case of a significant main effect in the two-way mixed ANOVA for the training groups only, a one-way ANOVA with Least Significant Difference (LSD) post-hoc test was performed to examine changes in variables over time for each training group. Where appropriate, Cohen's  $d$  was calculated to quantify the magnitude of difference within or between subjects. In the case of a within-subjects factor, it was corrected for dependence between means using the equation suggested by Morris and DeShon (2002);  $d = M_{\text{diff}} / SD_{\text{pooled}} \sqrt{2(1 - r)}$ , where  $M_{\text{diff}}$  is

mean difference between conditions,  $SD_{\text{pooled}}$  is pooled standard deviation, and  $r$  is correlation between means. Cohen's effect size was defined as follows:  $d < 0.2$  trivial effect, 0.2 - 0.5 small effect, 0.6 - 1.1 moderate effect and 1.2 – 1.9 as a large effect (Cohen 1992). All statistics were run on IBM® SPSS® Version 22.0 for Windows and the level of significance was set at  $P < 0.05$ .

## **5.3 Results**

### *5.3.1 Blood pressure and anthropometric measures*

There was no change in blood pressure or body composition following 9 weeks of HIT or in the control group (Table 5.1).

**Table 5.1** Resting measures before and after the experimental period

	15TG (n = 9)		30TG (n = 8)		CON (n = 8)	
	Pre	Post	Pre	Post	Pre	Post
Age (years)	27.0 ± 2.7	-	27.5 ± 4.5	-	25.9 ± 5.1	-
Height (cm)	177.4 ± 10.4	-	174.6 ± 9.5	-	174.8 ± 8.6	-
Body mass (kg)	77.1 ± 18.6	76.7 ± 17.5	71.8 ± 13.6	70.8 ± 11.8	76.1 ± 14.2	76.5 ± 13.8
Fat mass (kg)	13.3 ± 7.9	13.2 ± 8.2	11.0 ± 5.9	10.8 ± 6.4	13.1 ± 7.4	13.2 ± 7.2
Lean body mass (kg)	63.8 ± 15.1	63.5 ± 14.6	60.8 ± 16.3	60.0 ± 15.4	63.0 ± 13.2	63.3 ± 13.0
SBP (mm Hg)	123 ± 11	120 ± 9	121 ± 15	123 ± 11	127 ± 8	129 ± 7
DBP (mm Hg)	73 ± 8	75 ± 7	73 ± 11	76 ± 7	76 ± 8	77 ± 7
MAP (mm Hg)	87 ± 8	88 ± 9	86 ± 15	87 ± 6	91 ± 8	89 ± 8
PP (mm Hg)	49 ± 12	45 ± 6	48 ± 10	47 ± 9	51 ± 5	52 ± 5
HR rest (beats·min <sup>-1</sup> )	63 ± 13	63 ± 8	64 ± 11	59 ± 8	61 ± 12	57 ± 12

Values are means ± SD. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure. A 2-way analysis of variance with between (group) and repeated (time) factors showed no main effect of time, time by group interaction effect or main difference between groups in all resting measures.

### 5.3.2 Performance measurements

All performance measures were similar in all groups at baseline (Table 5.2). Both training groups significantly improved relative  $\dot{V}O_{2peak}$  (15TG: 12.1%,  $d = 1.77$ ,  $P < 0.001$ ; 30TG: 12.8%,  $d = 1.27$ ,  $P < 0.05$ ), absolute  $\dot{V}O_{2peak}$  (15TG: 11.1%,  $d = 2.79$ ,  $P < 0.001$ ; 30TG: 10.6%,  $d = 1.09$ ,  $P < 0.05$ ),  $O_2$  pulse (15TG: 10.5%,  $d = 1.64$ ,  $P < 0.01$ ; 30TG: 10.8%,  $d = 1.05$ ,  $P < 0.05$ ), time to exhaustion (15TG: 16.2%,  $d = 2.17$ ,  $P < 0.001$ ; 30TG: 12.8%,  $d = 1.70$ ,  $P < 0.01$ ),  $P_{max}$  (15TG: 13.8%,  $d = 2.23$ ,  $P < 0.001$ ; 30TG: 10.9%,  $d = 1.69$ ,  $P < 0.01$ ) and 10-km cycling time-trial performance (15TG: 8.6%,  $d = 3.47$ ,  $P < 0.01$ ; 30TG: 7.2%,  $d =$

0.86,  $P < 0.05$ ), while only 15TG increased critical power (7.8%,  $d = 0.87$ ,  $P < 0.05$ ) (Table 5.2). Although 30TG also increased critical power to a similar extent (7.4%,  $d = 0.67$ ), it did not reach a statistical significance ( $P = 0.11$ , Table 5.2). Crossover point, relative intensity at COP and HRmax were not significantly changed with 9 weeks of HIT (Table 5.2). All performance measures were not altered in CON following 9 weeks (Table 5.2).

**Table 5.2** Performance measures before and after the experimental period

	15TG (n = 9)		30TG (n = 8)		CON (n = 8)	
	Pre	Post	Pre	Post	Pre	Post
$\dot{V}O_{2\text{peak}}$ ( $\text{ml}^{-1} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ )* <sup>###</sup>	42.2 ± 5.4	47.3 ± 5.7 <sup>†††</sup>	40.6 ± 9.6	45.8 ± 7.9 <sup>†</sup>	47.4 ± 7.9	44.0 ± 5.8
$\dot{V}O_{2\text{peak}}$ ( $\text{l}^{-1} \cdot \text{min}^{-1}$ )* <sup>###</sup>	3.25 ± 0.84	3.61 ± 0.79 <sup>†††</sup>	2.92 ± 0.83	3.23 ± 0.65 <sup>†</sup>	3.59 ± 0.73	3.33 ± 0.57
HR max ( $\text{beats} \cdot \text{min}^{-1}$ )	180 ± 7	182 ± 6	178 ± 8	179 ± 6	186 ± 7	179 ± 11
$O_2$ pulse ( $\text{ml} \cdot \text{beat}^{-1} \cdot \text{kg}^{-1}$ )* <sup>##</sup>	23.7 ± 3.6	26.2 ± 2.9 <sup>††</sup>	23.2 ± 5.1	25.7 ± 4.7 <sup>†</sup>	25.7 ± 4.2	24.6 ± 2.9
Time to exhaustion (sec) <sup>***</sup> <sup>###</sup>	978 ± 205	1136 ± 264 <sup>†††</sup>	954 ± 280	1076 ± 283 <sup>††</sup>	1037 ± 221	1014 ± 216
Pmax (watts) <sup>***</sup> <sup>###</sup>	225 ± 40	256 ± 51 <sup>†††</sup>	220 ± 55	244 ± 55 <sup>††</sup>	237 ± 43	232 ± 42
Crossover point (watts)	62 ± 16	66 ± 12	67 ± 21	75 ± 15	62 ± 18	51 ± 26
Percentage of Pmax at COP (%)	28 ± 7	27 ± 6	31 ± 7	31 ± 6	27 ± 7	21 ± 9
Critical power (watts) <sup>**</sup>	218 ± 47	235 ± 49 <sup>†</sup>	204 ± 47	219 ± 52	219 ± 54	222 ± 55
10-km time trial (sec) <sup>***</sup>	977 ± 160	893 ± 112 <sup>††</sup>	969 ± 82	899 ± 89 <sup>†</sup>	912 ± 103	902 ± 112

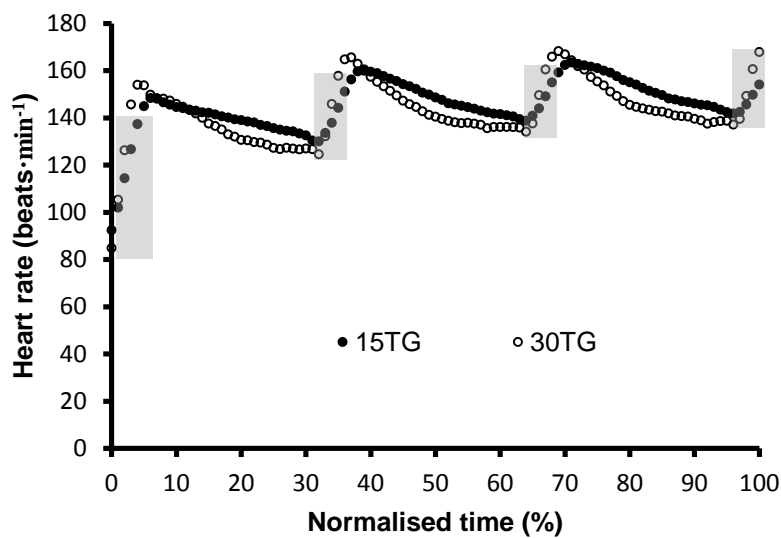
Values are means ± SD.  $\dot{V}O_{2\text{peak}}$ , peak oxygen uptake; Pmax, maximal power output; COP, crossover point. \*\*\*

Indicates  $P < 0.001$  for main effect of time. \*\* Indicates  $P < 0.01$  for main effect of time. \* Indicates  $P < 0.05$  for main effect of time. <sup>###</sup> Indicates  $P < 0.001$  for time by group interaction effect. <sup>##</sup> Indicates  $P < 0.01$  for time by group interaction effect. <sup>†††</sup> Indicates  $P < 0.001$  vs. pre within the same group. <sup>††</sup> Indicates  $P < 0.01$  vs. pre within the same group. <sup>†</sup> Indicates  $P < 0.05$  vs. pre within the same group. A 2-way mixed ANOVA was performed to determine main effect of time, time by group interaction effect and main difference between groups, whereas paired t-tests were employed to determine pre to post differences within the same group. In CON, Wilcoxon signed-rank tests were used to examine changes in critical power and 10-km time trial performance since the sample distributions obtained from the differences between paired scores were not assumed to be normal.

### 5.3.3 Heart rate and blood lactate responses during training sessions

There was no significant difference between the groups in heart rate during sprints or recovery, whereas only 30TG increased sprint HR with successive bouts (6 vs. 4 sprints,  $P < 0.01$ ,  $d = 1.35$ ; 6 vs. 5 sprints,  $P < 0.05$ ,  $d = 0.88$ , Table 5.3). Example of heart rate responses to each training protocol is shown below (Figure 5.1).

**Figure 5.1** Example of heart rate over 4 sprints and 3 rest periods (group mean)



HR is normalised to percentage of total time. Shaded areas indicate HR during sprints. Error bars are not shown for clarity.

Both training groups similarly increased blood lactate accumulation following four 15-s or 30-s sprints with the peak values observed immediately after sprint 4 (pre-sprint vs. peak post-sprint value; 15TG:  $P < 0.001$ ,  $d = 8.28$ , 30TG:  $P < 0.001$ ,  $d = 14.7$ , Table 5.3). Blood lactate concentration gradually decreased with time during the 10-min recovery phase in both groups (Table 5.3).

### 5. 3 Heart rate and blood lactate responses during the training

Physiological parameters	Training group	
Sprint heart rate (beats·min <sup>-1</sup> )**	15TG	30TG
average value over 4 sprints	135 ± 8	141 ± 11
average value over 5 sprints	138 ± 8	145 ± 11
average value over 6 sprints	139 ± 9	148 ± 12 <sup>††</sup> †
Recovery heart rate (beats·min <sup>-1</sup> )	15TG	30TG
average value over 3 rest periods	147 ± 8	143 ± 13
average value over 4 rest periods	149 ± 8	145 ± 13
average value over 5 rest periods	149 ± 8	149 ± 14
Blood lactate (mmol·l <sup>-1</sup> )***	15TG	30TG
Pre-sprint	1.5 ± 0.4	1.9 ± 0.2
0-min post-sprint 4	13.4 ± 2.1 <sup>aaa</sup> bb	14.0 ± 1.0 <sup>aaa</sup> b c
3-min post	13.2 ± 1.4 <sup>aaa</sup> bb	13.8 ± 1.2 <sup>aaa</sup> b c
5-min post	12.6 ± 1.8 <sup>aaa</sup> bb	13.1 ± 1.1 <sup>aaa</sup>
8-min post	12.4 ± 1.8 <sup>aaa</sup> b	13.0 ± 1.2 <sup>aaa</sup>
10-min post	11.4 ± 2.4 <sup>aaa</sup>	12.6 ± 1.8 <sup>aaa</sup>

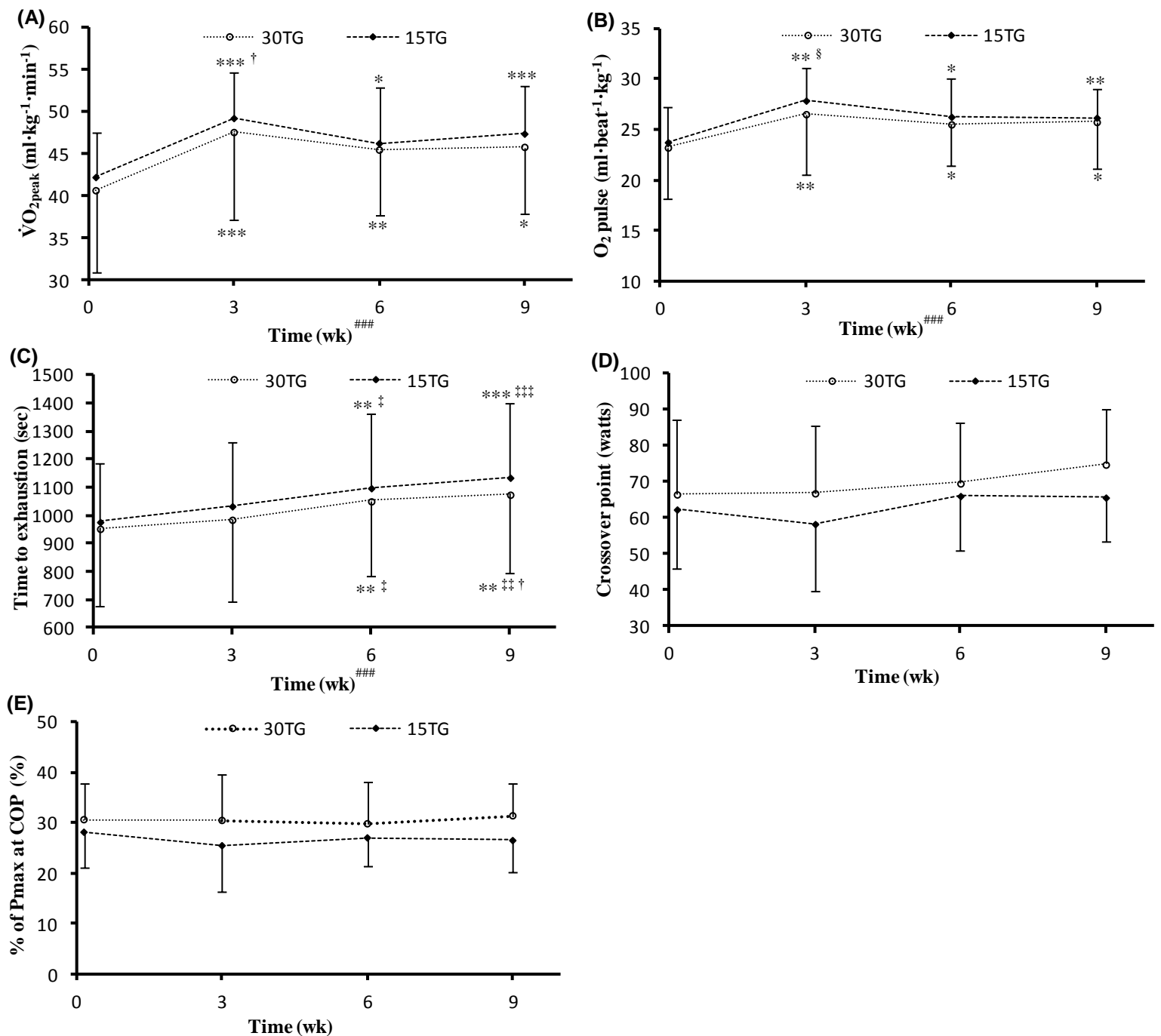
Values are means ± SD. \*\* Indicates P < 0.01 for main effect of sprint number. \*\*\*Indicates P < 0.001 for main effect of time. <sup>††</sup> Indicates P < 0.01 vs. 4 sprints within the same group. <sup>†</sup>Indicates P < 0.05 vs. 5 sprints within the same group. <sup>aaa</sup> Indicates P < 0.001 vs. pre-sprint within the same group. <sup>bb</sup> Indicates P < 0.01 vs. 10-min post within the same group. <sup>b</sup> Indicates P < 0.05 vs. 10-min post within the same group. <sup>c</sup> Indicates P < 0.05 vs. 5-min post within the same group. A 2-way mixed ANOVA showed no interaction effect or main difference between groups in either heart rate or blood lactate values. Changes in the variables over time for each group were determined via a 1-way ANOVA with LSD post-hoc test.

#### *5.3.4 Time course of changes in $\dot{V}O_{2peak}$ , $O_2$ pulse, time to exhaustion, crossover point and the relative intensity at COP*

$\dot{V}O_{2peak}$  rapidly increased with both training protocols and the highest values were observed at week 3 in both groups (15TG:  $49.2 \pm 5.4 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ , 30TG:  $47.5 \pm 10.3 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ , Figure 5.2A), indicating that the gain of  $\dot{V}O_{2peak}$  plateaued after 3 weeks. Likewise, the highest  $O_2$  pulse was observed following 3 weeks of the training in both groups (15TG:  $27.9 \pm 3.2 \text{ ml} \cdot \text{beat}^{-1} \cdot \text{kg}^{-1}$ , 30TG:  $26.5 \pm 6.0 \text{ ml} \cdot \text{beat}^{-1} \cdot \text{kg}^{-1}$ , Figure 5.2B). On the other hand, time to exhaustion was not significantly increased with training until week 6 and the greatest values were obtained at week 9 in both groups (15TG:  $1136 \pm 264 \text{ sec}$ , 30TG:  $1076 \pm 283 \text{ sec}$ , Figure 5.2C). Neither group significantly changed crossover point or percentage of  $P_{max}$  at COP over the course of 9 weeks (Figure 5.2 D&E).



**Figure 5.2** Time course of changes in peak oxygen uptake (A), peak O<sub>2</sub> pulse (B), time to exhaustion (C), crossover point (D) and relative intensity at crossover point (E) during the incremental test



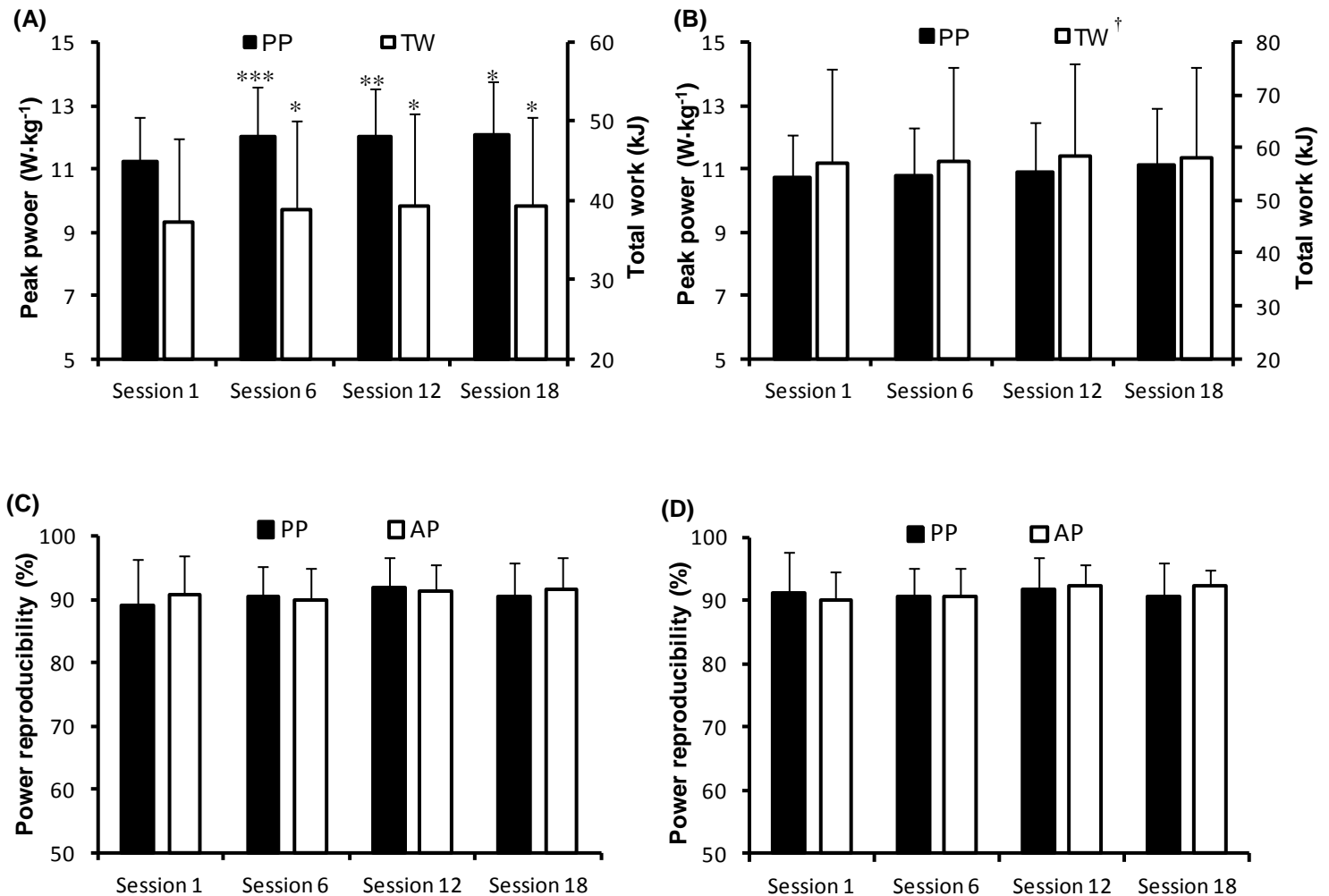
Pmax, maximal power output; COP, crossover point. ### Indicates  $P < 0.001$  for main effect of time. \*\*\* Indicates  $P < 0.001$  vs. baseline within the same group. \*\* Indicates  $P < 0.01$  vs. baseline within the same group. \* Indicates  $P < 0.05$  vs. pre within the same group. † Indicates  $P < 0.05$  vs. week 6 within the same group. ‡ Indicates  $P < 0.001$  vs. week 3 within the same group. ‡‡ Indicates  $P < 0.01$  vs. week 3 within the same group. ‡‡‡ Indicates  $P < 0.05$  vs. week 3 within the same group. § Indicates  $P < 0.05$  vs. week 9 within the same group.

A 2-way mixed ANOVA showed no interaction effect or main difference between groups in all variables. A 1-way ANOVA with LSD post-hoc test was performed to determine changes in the variables over time for each group.

### *5.3.5 The time course of changes in repeated sprint performance over 4 sprints*

30TG did not improve any measurements of repeated sprint performance over 9 weeks (Figure 5.3B), whereas 15TG significantly increased peak power and total work over the first 4 sprints during the sessions 6, 12 and 18 compared with the first session (Figure 5.3A). Percent of changes from the session 1 in 15TG were 7.0% ( $d = 2.12$ ,  $P < 0.001$ ), 7.4% ( $d = 1.60$ ,  $P < 0.01$ ) and 7.9% ( $d = 1.08$ ,  $P < 0.05$ ) in peak power, and 4.6% ( $d = 0.78$ ,  $P < 0.05$ ), 5.4% ( $d = 0.86$ ,  $P < 0.05$ ) and 5.4% ( $d = 0.77$ ,  $P < 0.05$ ) in total work for the sessions 6, 12 and 18, respectively (Figure 5.3A). Total work was greater in 30TG compared to 15TG (15TG vs. 30TG:  $38.6 \pm 11.2$  vs.  $57.8 \pm 17.6$  kJ,  $P < 0.05$ ,  $d = 1.33$ , Figure 5.3 A&B), whereas there was no difference between the groups in peak power or the reproducibility of peak and average power (Figure 5.3 A-D).

**Figure 5.3** Peak power and total work in 15TG (A) and 30TG (B) and the reproducibility of power in 15TG (C) and 30TG (D) during the first 4 sprints in the 1<sup>st</sup>, 6<sup>th</sup>, 12<sup>th</sup> and 18<sup>th</sup> training sessions



†Indicates that TW is greater than 15TG ( $P < 0.05$ ). \*\*\* Indicates  $P < 0.01$  vs. session 1 within the same group.

\*\* Indicates  $P < 0.01$  vs. session 1 within the same group. \* Indicates  $P < 0.05$  vs. session 1 within the same group. A 2-way mixed ANOVA showed a main difference in total work between the groups, whereas a 1-way ANOVA with LSD post-hoc test demonstrated the improvements in sprint performance in 15TG.

## 5.4 Discussion

The present study demonstrated divergent effects of sprint interval training on physiological and performance adaptations. While the gain in  $\dot{V}O_{2peak}$  reached a plateau following 3 weeks, time to exhaustion kept increasing until the end of the study in both 15-s and 30-s training

groups. In addition, reducing sprint duration did not diminish overall training adaptations, and indeed, only 15TG significantly increased critical power and repeated sprint performance. Considering that both sprint protocols resulted in a similar metabolic demand during the training (Table 5.3), sprint-to-rest ratio rather than duration would be important for bringing about training benefits.

#### *5.4.1 Performance measures in the incremental test*

Both training groups increased  $\dot{V}O_{2peak}$  to a similar extent following 9 weeks of training (12.1 and 12.8% for 15TG and 30TG, respectively), however, the greatest gains were observed at week 3 in both groups (16.6 and 17.0% for 15TG and 30TG, respectively). Similar to the current study, whilst Burgomaster et al. (2008) saw a 7.3% improvement in  $\dot{V}O_{2peak}$  following 3 weeks of Wingate-based HIT, it remained unchanged with additional 3 weeks of the training. Given that both studies increased the number of sprints performed with time, it seems that increasing sprint repetitions does not necessarily provide a further improvement in cardiorespiratory function. The levelling-off of  $\dot{V}O_{2peak}$  improvement observed in the current study might reflect that of cardiac adaptations. Using Sprague-Dawley rats, Wisløff et al. (2001) demonstrated that the majority of the increase in  $\dot{V}O_{2max}$  occurred within 4 weeks and time course of changes in  $\dot{V}O_{2max}$  reflected that of cardiac adaptations such as improved myocardial contractility and  $Ca^{2+}$  - handling in response to high-intensity aerobic interval training consisting of repeated 8-min runs at 85-90%  $\dot{V}O_{2max}$  alternated with 2 min of active recovery at 50-60%  $\dot{V}O_{2max}$ . Likewise, Kemi et al. (2004) employed the same high-intensity training protocol as Wisløff et al. (2001) and showed close time-dependent relationships between improvements in  $\dot{V}O_{2max}$  and those in myocardial hypertrophy and contractile function, and changes in both  $\dot{V}O_{2max}$  and myocardial function reached a plateau within 6 to 8

weeks. In the present study, cardiac function was not directly measured but estimated by  $\dot{V}O_{2\text{peak}}$  and  $\dot{V}O_{2\text{peak}}$  pulse and the time course of changes in  $\dot{V}O_{2\text{peak}}$  and  $\dot{V}O_{2\text{peak}}$  pulse showed a similar trend (Figure 5.2 A&B). Oxygen pulse is an indicator of both central (stroke volume) and peripheral (arterial-venous  $O_2$  difference) factors (Lavie et al. 2004). Therefore, the increase in  $\dot{V}O_{2\text{peak}}$  pulse cannot be totally attributed to central adaptation; however, previous studies that showed improved cardiac function also saw an increase in  $\dot{V}O_{2\text{peak}}$  pulse. Trilk et al. (2011) saw increases in stroke volume,  $\dot{V}O_{2\text{max}}$  and maximal  $\dot{V}O_{2\text{peak}}$  pulse but not in arterial-venous  $O_2$  difference in their overweight/obese female participants (baseline  $\dot{V}O_{2\text{max}}$ :  $21.6 \pm 1.1 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) following 4 weeks of Wingate-based HIT, suggesting that the gain in  $\dot{V}O_{2\text{peak}}$  pulse would reflect central improvement. Moreover, Wisløff et al. (2001) observed an increased  $\dot{V}O_{2\text{peak}}$  pulse across different exercise intensities (i.e. from sub-maximal to maximal) in their trained rats in addition to the improvements in  $\dot{V}O_{2\text{max}}$  and myocardial contractility. Therefore, it seems that the plateau of  $\dot{V}O_{2\text{peak}}$  gain observed in the current study is at least partially explained by that of cardiac adaptation. Although Macpherson et al. (2011) did not see an increase in maximal cardiac output/stroke volume despite increased maximal arterial-venous  $O_2$  difference and  $\dot{V}O_{2\text{max}}$  following 6 weeks of Wingate-based HIT in their recreationally active participants (baseline  $\dot{V}O_{2\text{max}}$ :  $46.8 \pm 5.1 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ), recovery modality (40%  $\dot{V}O_{2\text{peak}}$ ) employed in the current study might have facilitated a central adaptation due to an increased stroke volume/cardiac output during recovery (Takahashi and Miyamoto 1998; Crisafulli et al. 2004). Indeed, HR remained elevated in both training groups during the recovery periods (Table 5.3 & Figure 5.1), indicating that a greater cardiac demand has been achieved compared to the previous Wingate-based studies employing passive or very light recovery intensity (< 30W) (Burgomaster et al. 2005, 2006; Gibala et al. 2006; Hazell et al. 2010; Macpherson et al. 2011; Zelt et al. 2014). Nevertheless, none of the resting cardiovascular measures (resting HR and blood pressure measurements) were altered in either training group

(Table 5.1) which is in line with the study by Astorino et al. (2012) who also observed an improvement in peak  $\text{O}_2$  pulse but not in resting HR or blood pressure after 2 weeks of Wingate-based HIT. Likewise, whilst Esfandiari et al. (2013) saw increased exercise stroke volume and cardiac output due to increases in end-diastolic volume following 2 weeks of HIT consisting of 8 to 12 sets of 60-s cycling at 95 – 100%  $\dot{\text{V}}\text{O}_{2\text{max}}$  interspersed with 75 sec of rest, resting left ventricular function (e.g. cardiac output, stroke volume, end-diastolic volume), was not altered. On the other hand, in addition to an increased  $\dot{\text{V}}\text{O}_{2\text{max}}$ , Matsuo et al. (2014) observed gains in left ventricular mass, and resting stroke volume and heart rate following 40 sessions of sprint interval training (SIT) consisting of 7 sets of 30-s cycling at 120% $\dot{\text{V}}\text{O}_{2\text{max}}$  separated by 15-s rests or high-intensity aerobic interval training (HIAT) consisting of 3 sets of 3-min cycling at 80 to 90% $\dot{\text{V}}\text{O}_{2\text{max}}$  interspersed with 2-min recovery at 50%  $\dot{\text{V}}\text{O}_{2\text{max}}$ . This may suggest that when performing HIT, longer exercise duration or shorter rest duration would promote an increased aerobic demand, resulting in greater central adaptations. Indeed, average heart rates in SIT (161 beats·min<sup>-1</sup>) and HIAT (170 beats·min<sup>-1</sup>) in the study by Matsuo et al. (2014) were greater than those seen in 15TG or 30TG in the current study (Table 5.3). Further research is required to determine the effects of exercise duration or work: rest ratio on the morphological and functional adaptations of the heart via direct measurements (e.g. cardiac MRI, Doppler echocardiography) (Matsuo et al. 2014; Esfandiari et al. 2014).

In contrast to  $\dot{\text{V}}\text{O}_{2\text{peak}}$ , time to exhaustion showed a trend to be increased until the end of the study in both training groups (Figure 5.1C). Previously, increases in time to exhaustion were associated with improvements in mitochondrial function following 2 weeks of Wingate-based HIT (Burgomaster et al. 2005) or 8 weeks of high-intensity interval aerobic training at ~ 90%  $\dot{\text{V}}\text{O}_{2\text{max}}$  (Daussin et al. 2008). Daussin et al. (2008) also associated the increased TTE with

faster  $\dot{V}O_2$  kinetics resulting from an enhanced muscle  $O_2$  uptake. Likewise, Bailey et al. (2009) showed accelerated  $\dot{V}O_2$  kinetics and muscle  $O_2$  extraction during moderate- and severe-intensity exercise following 2 weeks of Wingate-based HIT. Accelerated  $\dot{V}O_2$  kinetics and muscle  $O_2$  uptake may delay the onset of depletion of muscle high-energy phosphates (e.g. PCr) and accumulation of fatigue related metabolites (e.g.  $H^+$ ,  $P_i$ ) (Jones et al. 2008; Vanhatalo et al. 2010), which likely results in an enhanced exercise tolerance (Bailey et al. 2009). Moreover, Daussin et al. (2008) also demonstrated an increased TTE in their continuous training group (20 to 35 min cycling at  $\sim 61\% \dot{V}O_{2max}$ ) in the absence of mitochondrial adaptation but due to greater improvements in capillary density and vascular conductance compared to the high-intensity training group. This indicates that improved muscle perfusion and thus  $O_2$  supply in addition to enhanced muscle  $O_2$  uptake may increase tolerable exercise duration (Daussin et al. 2008). Following 4 weeks of high-intensity training consisting of repeated one-legged knee extensor exercise at 150% of leg  $\dot{V}O_{2max}$ , there was an increased capillary density in both type I and II muscle fibres (Jensen et al. 2004). Therefore, it is possible that improvements in capillary density following HIT as well as mitochondrial adaptations are regulating the improvement in time to exhaustion. Taken together, it seems that peripheral adaptations mainly account for improvements in exercise tolerance with HIT and increasing sprint number may play a role in continuous improvements in tolerable exercise duration.

Crossover point is regarded as the work rate at which CHO-derived fuels become predominant over those derived from lipids for energy production (Brooks and Mercier 1994; Brooks 1997). Therefore, an increase in COP would suggest an increased fat oxidation rate or altered substrate utilisation during exercise. Crossover point and relative intensity at COP did not change throughout 9 weeks in both training groups (Figure 5.2 E&F). There have been

mixed findings regarding changes in substrate utilisation in response to Wingate-based HIT (MacDougall et al. 1998; Burgomaster et al. 2006, 2007, 2008). Burgomaster et al. (2008) saw an increase in the maximal activity of 3-hydroxyacyl CoA dehydrogenase ( $\beta$ -HAD, a mitochondrial marker for lipid oxidation) in addition to increased and decreased rates of whole-body fat and carbohydrate oxidation during 60-min exercise at 65% of pre-training  $\dot{V}O_{2\text{peak}}$  following 6 weeks of Wingate-based HIT. On the other hand, other Wingate-based studies failed to increase the maximal activity of  $\beta$ -HAD or the content of proteins associated with fatty acid transport such as fatty acid translocase (FAT/CD36) and plasma membrane-associated fatty acid binding protein (FABPpm) following 2 weeks (Burgomaster et al. 2006), 6 weeks (Burgomaster et al. 2007) or 7 weeks (MacDougall et al. 1998) of Wingate-based HIT. In contrast, Talanian et al. (2007) demonstrated that as few as 7 sessions of high-intensity aerobic interval training consisting of ten 4-min cycling at  $\sim 90\%$   $\dot{V}O_{2\text{peak}}$  interspersed with 2 min of recovery over 2 weeks increased the muscle protein content of FABPpm, the maximal activity of  $\beta$ -HAD and whole-body fat oxidation during 60-min cycling at 60% of pre-training  $\dot{V}O_{2\text{peak}}$ . Considering that exercise duration of the training protocol employed by Talanian et al. (2007) (40 min per session) is far greater than that of typical Wingate-based HIT (2 to 3 min of all-out efforts per session), total exercise volume may play an important role in improving fat metabolism when performing HIT (Gibala and McGee 2008). Whilst the current study was conducted over 9 weeks, both groups performed their respective training twice per week (18 sessions in total) which is equivalent to three sessions per week over 6 weeks as employed by Burgomaster et al. (2007, 2008). Therefore, although it is currently unknown in terms of minimum volume of HIT to increase the capacity for fat oxidation, training frequency as well as intervention may need to be increased to see robust improvements in fat metabolism with Wingate-based HIT.



#### *5.4.2 Effects of sprint duration on endurance and repeated sprint performance*

Reducing sprint duration did not diminish exercise performance adaptations, and the findings of the current study reinforce previous work that also showed no difference in performance adaptations such as 5-km cycling time trial or critical power when sprint duration was reduced from 30 to 10 sec (Hazell et al. 2010) or 15 sec (Zelt et al. 2014). In the current study, similar magnitude of improvement was seen in the two training groups in 10-km time trial (15TG vs. 30TG: 8.6 vs. 7.2%) and critical power (15TG vs. 30TG: 7.8 vs. 7.4%) (Table 5.2). Although changes in critical power did not reach a statistical significance in 30TG, the magnitude of gain was higher than that (5.2%) seen in 30-s Wingate-based HIT group in the study by Zelt et al. (2014). Whilst eight of nine participants increased CP following 9 weeks in 15TG, two of eight participants decreased it in 30TG. Therefore, inter-individual variability may have precluded a statistical significance. A high level of power production during all-out sprinting would require an increased level of muscle fibre recruitment (Esbjornsson-Liljedahl et al. 2002) and rapid training gains derived from sprint interval training has been linked to the high degree of stress to working muscles, in particular to type II muscle fibres (Hazell et al. 2010; Buchheit et al. 2012; Sloth et al. 2013). In this study, sprint: rest ratio was matched between the training groups (1:8) as opposed to the study by Hazell et al. (2010) or Zelt et al. (2014) and as a result, there was no difference in the reproducibility of power production during the training between the groups (Figure 5.3 C&D). Moreover, although sprint duration was reduced by 50% in 15TG, training volume (total work) was only reduced to ~ 67% of that obtained in 30TG (Figure 5.3 A&B). This indicates that the majority of work achieved in a 30-s sprint is produced during the initial phases as previously reported (Bogdanis et al. 1996a, 1996b), which may negate the need for performing a prolonged sprint. The choice of sprint duration (15 seconds) was made based on the previous studies showing that the majority of anaerobic metabolism (i.e. the degradation

of phosphocreatine and glycogen) occurs within the first 15 sec when performing a maximal sprint (Bogdanis et al. 1996a, 1998; Parolin et al. 1999). Similar level of blood lactate accumulation was recorded between the groups (Table 5.3), which would support a similar anaerobic demand of both sprint protocols. Moreover, whilst heart rate during sprints tended to be increased in 30TG compared to 15TG, HR during recovery showed an opposite trend (Table 5.3 & Figure 5.1), indicating that when sprint: rest ratio is matched, overall aerobic demand is not affected by sprint duration.

Improvements in repeated sprint performance during training intervention were only seen in 15TG (Figure 5.3). 30TG did not improve any of the sprint performance measures over the 4 sprints throughout the study period, which is not in line with previous Wingate-based studies that report improvements in performance during a single or repeated 30-s Wingate tests (MacDougall et al. 1998; Burgomaster et al. 2005, 2006; Hazell et al. 2010; Whyte et al. 2010; Astorino et al. 2012). Nevertheless, Whyte et al. (2010) did not observe a gain in peak power during a single Wingate test following 2 weeks of Wingate-based HIT, and mean power or total work during repeated Wingate tests was not increased with 4 weeks (Trilk et al. 2011) or 2 weeks (Burgomaster et al. 2005) of Wingate-based HIT. Prolonged sprint duration ( $\geq 30$  sec) may have less impact on improvements in anaerobic metabolism and thus sprint performance. Barnett et al. (2004) observed no changes in glycolytic enzyme (phosphofructokinase, PFK) activity or muscle fibre type distribution following 8 weeks of sprint interval training consisting of 3 to 6 30-s cycling sprints separated by 3 min of passive recovery (similar to Wingate-based HIT). Although MacDougall et al. (1998) observed increased activities of PFK and hexokinase (Hex) following 7 weeks of Wingate-based HIT, no changes were observed in other glycolytic enzyme activities such as glycogen phosphorylase (PHOS) and lactate dehydrogenase (LDH). In contrast, more robust increases

in glycolytic enzyme activities have been reported when sprint duration is short ( $\leq 15$  sec) or 30-s sprints were interspersed with longer recovery (12 min) (Linossier et al. 1997; Dawson et al. 1998; Parra et al. 2000; Rodas et al. 2000). Furthermore, Dawson et al. (1998) showed an increased portion of type II fibres following 6 weeks of sprint interval training consisting of repeated 40 to 60-m sprints which was associated with improved sprint performance (40-m sprint time). Nevertheless, changes in glycolytic enzyme activities have not been correlated with sprint performance (Dawson et al. 1998; Para et al. 2000; Rodas et al. 2000; Barnett et al. 2004), and as reflected by heart rate and blood lactate accumulation during training (Table 5.3 & Figure 5.1), it seems that similar aerobic and anaerobic metabolism were achieved between the training groups. Therefore, it is unlikely that there was a significant difference in metabolic or morphological adaptations between the groups. Prior knowledge of sprint number or prolonged sprint duration has been shown to induce anticipatory pacing strategy, resulting in reduced neuromuscular activity and power generation (St Clair Gibson et al. 2001; Ansley et al. 2004; Billaut et al. 2011). Sprint performance was assessed during the training intervention and the number of sprint was increased every 3 weeks (every 6 sessions) in the current study and the lack of improvement seen in 30TG might be explained by the adoption of pacing strategy due to longer sprint duration as well as increased sprint repetitions.

## **5.5 Conclusions**

The novel findings of the current study are that there are divergent effects of sprint interval training on the time course of physiological and performance adaptations, and that reducing sprint duration does not diminish overall aerobic adaptations, provided that sprint-to-rest ratio is fixed (i.e. 1:8). Physiological and performance improvements induced by Wingate-based HIT has been shown to be comparable to those derived from traditional endurance training

lasting more than 60 min (Gibala et al. 2006; Burgomaster et al. 2008). The present study further demonstrated that only 50% of total time commitment is required to gain similar or even greater training benefits compared to typical Wingate-based HIT. This indicates that sprint-to-rest ratio rather than sprint duration would be important for inducing training adaptations, and that individuals can improve their cardiorespiratory function and endurance performance by performing HIT requiring only 7 to 11.5 minutes in total (i.e. including recovery periods). Nevertheless, while both training groups increased time to exhaustion towards the end of the study, the improvements in  $\dot{V}O_{2peak}$  plateaued following 3 weeks despite the increase in sprint number, suggesting that training stimulus needs to be altered through a different strategy to see continuous cardiorespiratory improvements. In this study, recovery intensity (40%  $\dot{V}O_{2peak}$ ) was kept constant throughout the study period; however, gradual increase in recovery intensity or reduction in recovery duration may be required to ensure a progressive cardiorespiratory overload (Kavaliauskas et al. 2015).

# **Chapter 6**

## **General Discussion**

## 6.1 Introduction

Main aims of this PhD were to determine the influence of recovery intensity on physiological and performance adaptations to sprint interval training, and the time course of training adaptations to such training. Despite the increased utilisation of sprint interval training to promote physiological and metabolic improvements, little attention has been paid to workload during recovery intervals. It is plausible that recovery intensity could alter the adaptations to this type of training. Furthermore, the majority of previous studies employing sprint interval training have been conducted over a relatively short timeframe (2 to 6 weeks), and therefore little is known regarding the time course of physiological and performance adaptations in response to high-intensity training.

## 6.2 Summary of main findings and their interpretation

### 6.2.1 *The arrangement of recovery intensity during sprint interval training*

The findings derived from the studies 1 and 2 suggest that the arrangement of recovery intensity should be considered in order to maximise training adaptations to high-intensity interval training. In study 1, it has been demonstrated that active recovery at 30 to 40%  $\dot{V}O_{2peak}$  increases aerobic metabolism by 5 to 80% during typical Wingate-based HIT compared to passive recovery or lighter recovery intensity (20%  $\dot{V}O_{2peak}$ ), and subsequently 6 to 8% greater endurance performance adaptations were confirmed with active recovery (40%  $\dot{V}O_{2peak}$ ) in study 2. Although active but not trained individuals were recruited in study 2 (Table 4.2), Ben Abderrahman et al. (2013) have also demonstrated that repeated 30-s runs at 100 to 110%  $\dot{V}O_{2max}$  with active recovery (~50%  $\dot{V}O_{2max}$ ) improved  $\dot{V}O_{2max}$  by approximately 6% in well-trained subjects ( $\dot{V}O_{2max}$ : 59 ml·kg<sup>-1</sup>·min<sup>-1</sup>), which was not confirmed with passive recovery utilising the same HIT programme. Therefore, it seems that ~ 6 to 8% greater improvements in aerobic capacity or endurance performance would be ensured with

active recovery at 40 to 50%  $\dot{V}O_{2\max}$  when performing sprint interval training irrespective of the fitness level of individuals. Considering that cardiorespiratory variables ( $\dot{V}O_2$ ,  $\dot{V}CO_2$  and HR) during recovery, but not sprints, were increased by 5 to 38% with the active recovery in study 2 (Table 4.4), cardiorespiratory demand during recovery may play a role in aerobic adaptations to sprint interval training. In study 1,  $\dot{V}O_2$  was kept elevated during the 4-min recovery with active recovery (e.g. 40% recovery group had a rate of  $O_2$  consumption equivalent to 63%  $\dot{V}O_{2\text{peak}}$ ). Similarly, Bogdanis et al. (1996b) reported an increased  $\dot{V}O_2$  (corresponding to 55%  $\dot{V}O_{2\max}$ ) when their participants cycled at 40%  $\dot{V}O_{2\max}$  following a 30-s maximal sprint. This indicates that actual cardiorespiratory demand during recovery phase after all-out efforts would be greater than a targeted % $\dot{V}O_{2\max}$ . Furthermore, the arrangement of sprint duration or sprint-rest-ratio would also have an impact on aerobic metabolic demand during sprint interval training.

### *6.2.2 The influence of sprint duration and sprint-to-rest ratio on training adaptations*

In study 2, it has been demonstrated that when sprint duration is fixed (30-s), active recovery is more effective in inducing endurance adaptations compared with passive recovery due to an increased aerobic metabolism during training. In study 3, it has been shown that a 50% reduction in sprint duration does not diminish overall training adaptations when recovery intensity (40% of  $\dot{V}O_{2\max}$ ) and sprint-to-rest ratio (1:8) are matched. Considering that 4 to 6 15-sec sprints separated by 2 min of recovery only requires total time commitment of 7 to 11.5 min (i.e. 1 to 1.5 min of all-out efforts and 6 to 10 min of recovery), it would be a time-efficient alternative for the conventional Wingate-based HIT as well as traditional aerobic training to induce physiological and performance adaptations.

In contrast to the study by Hazell et al. (2010) who observed similar training adaptations when 10-s sprints were interspersed with either 2 or 4 min of recovery, Kavaliuskas et al.

(2015) have recently demonstrated that training adaptations are dependent on sprint-to-rest ratio utilising the same sprint duration (i.e. 10 sec). Whilst the greatest aerobic adaptations (improvements in  $\dot{V}O_{2peak}$ , time to exhaustion and 3-km run time trial) were achieved in the training group with shorter recovery (work: rest ratio: 1:3), improvements in anaerobic performance (peak and average power during a 30-s Wingate test) were greater when 10-s sprints were interspersed with longer rest duration (work: rest ratio: 1:8 to 1:12) (Kavaliauskas et al. 2015). Furthermore, aerobic adaptations were associated with heart rate responses during training and HR was increased with the reduction in recovery duration (Kavaliauskas et al. 2015), indicating that aerobic metabolic demand is influenced by sprint-to-rest ratio. In the study by Hazell et al. (2010), both 10-s sprint protocols were interspersed with relatively long recovery duration (10s: 2min or 10s: 4min; i.e. work: rest ratio of 1:12 or 1:24) and the additional 2-min recovery (i.e. 10s: 4min group) did not result in a further improvement in power reproducibility during the training. Although Hazell et al. (2010) did not report any cardiorespiratory variables or muscle and blood metabolites (e.g. lactate), this may suggest a similar metabolic demand between the groups, leading to comparable training gains (Hazell et al. 2010). Indeed, overall training aerobic (HR) and anaerobic (blood lactate) demands were not significantly different between 15TG and 30TG in study 3 (Table 5.3), resulting in similar power reproducibility between the groups (Figure 5.3C&D). Taken together, it seems that it is sprint-to-rest ratio rather than sprint duration that determines metabolic demands and intensity of training and as such would be important for determining training adaptations. If the main purpose of training is to improve aerobic capacity, then shorter sprint-to-rest ratio (e.g. 1:3) would be suitable, whereas sprint-to-rest ratio of 1:8 to 1:12 should be chosen if one wishes to improve both anaerobic and aerobic capacities (Hazell et al. 2010; Kavaliauskas et al. 2015)



### *6.2.3 Time course of gains in peak oxygen uptake in response to high-intensity training*

To date, limited data are available regarding the time course of changes in cardiorespiratory function in response to high-intensity exercise training. In line with Burgomaster et al. (2008), a plateau in  $\dot{V}O_{2\text{peak}}$  gain was observed after 3 weeks with both 15-s and 30-s training groups in study 3 (Figure 5.2A). Likewise, Astorino et al. (2013) saw the majority (60%) of gains in  $\dot{V}O_{2\text{max}}$  within 3 weeks and the improvement levelled off at 6 weeks when their participants performed HIT consisting of 6 to 10 x 60-s cycling at 80 to 90%  $\dot{V}O_{2\text{max}}$  interspersed with 75 s of active recovery (40W) over 12 weeks. Conversely, Matsuo et al. (2014) demonstrated continuous increase in  $\dot{V}O_{2\text{max}}$  throughout their 8-week study period in both sprint interval training (7 x 30-s cycling at 120%  $\dot{V}O_{2\text{max}}$  separated by 15-s rest) and high-intensity aerobic interval training (3 x 3-min cycling at 85 to 90%  $\dot{V}O_{2\text{max}}$  interspersed with 2 min of active recovery at 50%  $\dot{V}O_{2\text{max}}$ ). The short recovery periods (work: rest ratio: 2:1 or 1.5: 1) employed by Matsuo et al. (2014) may have provided greater stimulus for adaptations of cardiorespiratory function compared to the study by Astorino et al. (2013) or study 3 in this thesis. Indeed, Matsuo et al. (2014) saw greater session-averaged HR values in sprint interval training (161 bpm) and high-intensity aerobic interval training (170 bpm) compared to 15TG (143 bpm) or 30TG (145 bpm) in study 3. Both studies recruited participants with similar age group (~ 27 years) as well as baseline  $\dot{V}O_{2\text{max}}$  (~ 41 to 42 ml·kg<sup>-1</sup>·min<sup>-1</sup>), indicating that the differences in the HR values between the studies are mostly explained by the shorter recovery chosen by Matsuo et al. (2014). Therefore, as mentioned above, it seems that when designing a HIT programme, recovery intensity or work: rest ratio would be a key factor in determining cardiac demand and thus aerobic adaptations. In order for HIT to ensure sufficient cardiac demand and to continuously improve  $\dot{V}O_{2\text{max}}$ , a gradual

increase in recovery intensity (Ben Abderrahman et al. 2013) or decrease in recovery duration (Kavaliauskas et al. 2015) with the progress of training may be required.

#### *6.2.4 Time course of endurance performance adaptations to sprint interval training*

In addition to cardiorespiratory adaptations, the majority of endurance performance gains derived from Wingate-based HIT may occur within the initial weeks. In study 2, both ARG and PRG improved 10-km time trial performance by 8.6% and 6.7%, respectively following 2 weeks of HIT (Table 6.1). The magnitude of the improvements in the study is comparable to that observed in study 3 (15TG: 8.6%; 30TG: 7.2%, Table 6.1). Likewise, ARG improved critical power by 7.9% and such improvement is not different from those achieved by 15TG (7.8%) and 30TG (7.4%) in study 3 (Table 6.1). Furthermore, a similar degree of improvement (5 to 10%) in time trial performance ranging from 5 to 30 km is also reported following 2 to 6 weeks of Wingate-based HIT (Burgomaster et al. 2006, 2007; Gibala et al. 2006; Babraj et al. 2009; Hazell et al. 2010; Macpherson et al. 2011). Although less is known regarding effects of Wingate-based HIT on critical power, a 5 to 7% improvement in CP was seen following 4 weeks of Wingate-based HIT (Zelt et al. 2014), which is again similar to that observed in study 2 or 3 (7.4 to 7.9%). Previously, the improvements in time-trial performance have been associated with the increases in muscle oxidative capacities as reflected by increased mitochondrial enzymatic activity or content (e.g. cytochrome c oxidase, COX, citrate synthase, CS) (Burgomaster et al. 2006; Gibala et al. 2006). However, the majority of mitochondrial adaptations to sprint interval training may complete within a few weeks. A similar increase in the activity of CS (36 to 38%) has been reported following 2 weeks (Burgomaster et al. 2005) or 7 weeks (MacDougall et al. 1998) of Wingate-based HIT. Moreover, whilst Burgomaster et al. (2007) saw an increase in COX4 protein content within 1 week with Wingate-based HIT, it remained unchanged thereafter over the course of 6

weeks. Sprint interval training has been also shown to improve muscle buffering capacity (Gibala et al. 2006) or ionic regulation (McKenna et al. 1997b; Harmer et al. 2000; Mohr et al. 2007). Thus, improvements in endurance performance cannot be exclusively explained by mitochondrial adaptations; however, similar endurance performance adaptations seen in the previous Wingate-based studies (including the studies 2 and 3 in this PhD) over different timeframes may at least partially reflect early adaptations of muscle oxidative potential.

**Table 6.1 Summary of PhD studies**

Study1		
Purpose	To determine effects of recovery intensities on acute physiological responses	
Hypothesis	Aerobic metabolism would be increased with recovery intensity	
Protocol	4 x 30-sec cycle sprints against 7.5% bodyweight separated by 4 min of recovery	
Main Variables	% changes when compared with passive recovery	
$\dot{V}O_2$ -to-sprint work ratio (ml·min <sup>-1</sup> ·kJ <sup>-1</sup> )*	Increased by higher recovery intensities (30 and 40% $\dot{V}O_{2peak}$ ) only by ~ 15%	
Peak power in Sprint 4 (W·kg <sup>-1</sup> )	All active recovery conditions (20 to 40% $\dot{V}O_{2peak}$ ) increased it by ~ 6 to 12%	
AP drop rate relative to sprint 1 in S4 (%)	Higher recovery intensities (30 and 40% $\dot{V}O_{2peak}$ ) improved it by ~ 4 to 6%	
Study 2		
Purpose	To determine effects of recovery mode on training adaptations	
Hypothesis	Greater endurance adaptations would be achieved with active recovery	
Protocols	6 sessions of 4 to 6 30-s sprints with 4-min recovery over 2 weeks	
Main Variables	% changes following 2 weeks of HIT	
	Active recovery group (40% $\dot{V}O_{2peak}$ )	Passive recovery group
$\dot{V}O_{2peak}$ (ml·min <sup>-1</sup> ·kg <sup>-1</sup> )	no change	no change
10-km cycling time-trial (sec)	Improved by 8.6%	Improved by 6.7%
Time to exhaustion (sec)	Increased by 6.3%	no change
Critical power (W)	Increased by 7.9%	no change
Study 3		
Purpose	To determine effects of sprint duration and the time course of adaptations	
Hypothesis	No difference between training groups and early adaptations would be seen	
Protocols	18 sessions of 4 to 6 15-s or 30-s sprints with 2- or 4-min recovery over 9 weeks	
Main Variables	% changes following 9 weeks of HIT	
	15-s group	30-s group
$\dot{V}O_{2peak}$ (ml·min <sup>-1</sup> ·kg <sup>-1</sup> )	Increased by 12.1%	Increased by 12.8%
10-km cycling time-trial (sec)	Improved by 8.6%	Improved by 7.2%
Time to exhaustion (sec)	Increased by 16.2%	Increased by 12.8%
Critical power (W)	Increased by 7.8%	Increased by 7.4%
	Time course of changes over 9 weeks	
Peak oxygen uptake	Greatest after 3 weeks	Greatest after 3 weeks
Time to exhaustion	Greatest after 9 weeks	Greatest after 9 weeks
AP, average power; S4, sprint 4. *Average values over 4 sprints		

AP, average power; S4, sprint 4. \*Average values over 4 sprints

## 6.3 Consideration of experimental protocols

### 6.3.1 Incremental test to exhaustion

In study 3, improvements in both  $\dot{V}O_{2\text{peak}}$  and  $O_2$  pulse plateaued at 3 weeks in both training groups; however, this might be partially attributed to the increased time to exhaustion with training. Astorino et al. (2004) observed a 4% lower  $\dot{V}O_{2\text{max}}$  during an incremental running protocol to exhaustion lasting ~ 14 min compared to shorter protocols (7 to 10 min) in young active male and female participants varying in fitness level ( $\dot{V}O_{2\text{max}}$ :  $54.8 \pm 10.2 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ). Moreover, they also saw a lower  $O_2$  pulse in the long test protocol compared to the short protocols. In line with this study, McCole et al. (2001) showed a decreased stroke volume when time to exhaustion was increased from 6 min to 12 min during an incremental running test due to an increase in stage duration (from 1 min to 3 min). Likewise, Lepretre et al. (2004) saw a reduction in SV when tolerable duration was increased from approximately 5 min to 12 min during exhaustive cycling at constant work load either at 88% or 100%  $\dot{V}O_{2\text{max}}$ . The difference in SV between test protocols varying in exercise duration may be attributed to the degree of rise in core temperature (McCole et al. 2001; Astorino et al. 2004). The magnitude of rise in core temperature may be increased with an extended exercise duration, possibly resulting in cutaneous vasodilation and thus a reduction in venous return and SV (McCole et al. 2001; Astorino et al. 2004). In study 3, increment rate was kept constant (35W increase every 3 min) throughout the study; however, while time to exhaustion was not significantly increased at 3 weeks compared to baseline values in both training groups, it was significantly increased thereafter in both groups (i.e. 3 weeks > 6 weeks > 9 weeks). Thus, the slight decreases in  $\dot{V}O_{2\text{peak}}$  and  $O_2$  pulse seen at 6 and 9 weeks compared to those observed at 3 weeks in the third study (Figure 5.2A & 5.2B) might be partially explained by the increased tolerable exercise duration. In order to reduce the burden of participants,

cardiorespiratory performance and time to exhaustion were assessed during the same test in study 3. However, if the main purpose of a study is to evaluate effects of training intervention on cardiorespiratory adaptations, the length of testing protocol should be considered (McCole et al. 2001).

### *6.3.2 Repeated Wingate tests*

In the studies 2 and 3, improvements in repeated sprint performance were not confirmed in the 30-s training groups irrespective of recovery mode. However, this might be due to the method employed in these studies to assess performance. Both studies determined repeated sprint performance during the training intervention and total sprint number was not controlled between testing conditions. Although only the first 4 sprints were included for the analysis, the participants covered more sprints as the study progressed, which may have induced anticipatory pacing strategy during the initial sprints (Billaut et al. 2011). Likewise, Trilk et al. (2011) did not see the increase in total work over the first four 30-s Wingate sprints when total sprint number was not matched between conditions. Conversely, previous Wingate-based studies showing improved Wingate performance controlled total sprint number between the testing conditions (Burgomaster et al. 2005; Zelt et al. 2014) or employed a single 30-s Wingate test to assess anaerobic performance (Burgomaster et al. 2006; Gibala et al. 2006; Hazell et al. 2010; Astorino et al. 2012). Thus, it seems that to accurately assess changes in sprint performance with training intervention, total sprint number needs to be identical between conditions.

## 6.4 Future direction

### 6.4.1 Optimisation of recovery mode during high-intensity interval training

In study 2, 5 to 7% greater aerobic performance adaptations were observed with active recovery ( $40\% \dot{V}O_{2\text{peak}}$ ) following 2 weeks of Wingate-based HIT in healthy but not trained populations ( $\dot{V}O_{2\text{peak}}$ :  $37 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ). Similarly, Ben Abderrahman et al. (2013) have demonstrated that  $\dot{V}O_{2\text{max}}$  were only increased with active recovery ( $\sim 50\% \dot{V}O_{2\text{max}}$ ) following 7 weeks of high-intensity interval running training at 100 to 110%  $\dot{V}O_{2\text{max}}$  in well-trained subjects ( $\dot{V}O_{2\text{max}}$ :  $59 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ). This indicates that greater aerobic adaptations are achieved with active recovery when performing HIT regardless of fitness level of individuals or exercise mode (i.e. cycling or running). Nevertheless, due to less muscle groups involved in cycling, the degree of intramuscular forces in the working muscles during cycling would be greater compared with running (Lepretre et al. 2004). Therefore, the impact of recovery intensity on metabolic demand and thus overall training adaptations may be different according to exercise mode even in the same individuals.

Furthermore, since recovery intensity during HIT is typically determined relative to  $\dot{V}O_{2\text{max}}$  (Bogdanis et al. 1996b; Dupont et al. 2004, 2007; Spencer et al. 2008), testing protocols that determine  $\dot{V}O_{2\text{max}}$  should be considered. The maximal work rate that elicits  $\dot{V}O_{2\text{max}}$  has been shown to be dependent on the rate of work rate increase during a graded exercise test (Bishop et al. 1998; McCole et al. 2001; Astorino et al. 2004), and therefore, absolute recovery intensity (power output or velocity) at the identical relative intensity (e.g.  $50\% \dot{V}O_{2\text{max}}$ ) would be different even in the same individuals according to the protocols utilised to determine  $\dot{V}O_{2\text{max}}$ . Consequently, not only exercise mode but also the protocol of  $\dot{V}O_{2\text{max}}$  measurement likely determine the intensity of recovery during HIT and thus may influence overall training adaptations.

Active recovery was also utilised in study 3 and the intensity of recovery ( $40\% \dot{V}O_{2\text{peak}}$ ) was kept constant throughout 9 weeks. However, greater improvements in  $\dot{V}O_{2\text{peak}}$  or endurance performance might have been seen if the recovery intensity was gradually increased with time. Nevertheless, whilst higher recovery intensity may induce greater aerobic adaptations, exercise intensity (the level of power production) could be compromised with the increase in recovery intensity, which might result in a diminished adaptation of anaerobic performance (Kavaliauskas et al. 2015). It is currently unknown regarding the upper limit of recovery intensity which induces greater aerobic adaptations without a compromised anaerobic adaptation and thus further studies are required to address this issue.

#### *6.4.2 Number of sprints*

Whilst the majority of previous Wingate-based studies increased sprint number as the study progressed (Burgomaster et al. 2005, 2006, 2007, 2008; Gibala et al. 2006; Babraj et al. 2009; Whyte et al. 2009; Hazell et al. 2010; Trilk et al. 2011; Astorino et al. 2012; Zelt et al. 2014), it is currently unknown regarding the effects of total sprint number on training adaptations. It appears that increasing the number of sprint does not have an impact on cardiorespiratory adaptations. In study 3,  $\dot{V}O_{2\text{peak}}$  was not increased after 3 weeks despite the increase in sprint number. Likewise, Burgomaster et al. (2008) increased sprint number with time; however, the gains in  $\dot{V}O_{2\text{peak}}$  levelled off following 3 weeks. Whilst time to exhaustion was kept increasing towards the end of study 3 (Table 5.2C), it remains unclear whether the improvements are attributed to the increase in sprint number since no direct comparison was made between sprint training protocols with different total sprint number. However, it has been shown that muscle  $O_2$  extraction is increased with successive sprints during 6 x 30-s cycling sprints interspersed with 2 min of passive rest (Buchheit et al. 2012). This indicates that the increase in sprint number may provide further stimulus for aerobic energy system and greater number



of sprints (> 6 sprints) might be necessary to see continuous improvement in cardiorespiratory function or endurance exercise performance. On the other hand, if the goal of training is to maintain cardiorespiratory function or endurance performance, less sprint repetitions (e.g. two x 30-s sprints) might be required, which reduces total time commitment as well as the amount of all-out efforts and therefore should enhance the practicality of the training. Future studies should reveal an optimal number of sprints according to individuals' need.

#### *6.4.3 Resistive force*

In addition to recovery modality, resistive force during Wingate-based HIT have not been considered previously and the majority of studies utilised 7.5% of total body mass (TBM) (Burgomaster et al. 2005, 2006, 2007, 2008; Gibala et al. 2006; Babaj et al. 2009; Astorino et al. 2011, 2012; Zelt et al. 2014). However, as opposed to traditional resistive force (7.5% TBM), Hazell et al. (2010) employed a greater resistive load (10% of TBM) whereas Whyte et al. (2010) and Trilk et al. (2011) chose a smaller braking force (6.5% FFM or 5% TBM, respectively) during the training. Greater resistive force could be favourable to producing greater peak power compared to traditional resistance when young active adults perform all-out cycling sprints (MacIntosh et al. 2003; Baker et al. 2009), whereas smaller braking force seems to allow overweight/obese populations to generate better power (Baker and Davies 2006; Whyte et al. 2010; Trilk et al. 2011). Considering that exercise intensity (i.e. the level of power production) during sprint interval training has been associated with training gains (Bailey et al. 2009; Buchheit et al. 2012; Sloth et al. 2013), the selection of resistive force may have an impact on overall training adaptations. Although neither ARG nor PRG increased  $\dot{V}O_{2peak}$  in study 2, there was a tendency for male participants (n=4) in ARG to increase  $\dot{V}O_{2peak}$  whereas such trend was not seen in female participants (n=3) in the same

group. Three of the four male participants increased  $\dot{V}O_{2\text{peak}}$  from pre to post ( $40.3 \pm 5.5$  to  $41.8 \pm 4.6 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ,  $P = 0.086$ ), whereas none of the female participants improved it ( $32.8 \pm 3.7$  to  $31.2 \pm 5.9 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) following 2 weeks of HIT. This discrepancy could be attributed to the resistive force employed in the study (i.e. 7.5% TBM for both male and female participants), which resulted in a large difference in power generation between the sexes (e.g. female participants produced a 27% less peak power compared to male counterparts). In study 3, on the other hand, less difference was confirmed in peak power generation (15.7%) between the sexes due to the smaller resistive force (6.5% TBM) employed for the female participants, and consequently both training groups showed improvements in  $\dot{V}O_{2\text{peak}}$  irrespective of gender. Considering a large difference in body composition between the sexes (Billaut and Bishop 2009) or among individuals, adopting resistive force relative to fat free mass rather than total body mass may reduce inter-individual difference of adaptations to sprint interval training (Baker and Davis 2006; Baker et al. 2009). Moreover, since resistive force was kept constant throughout the study period in all of the previous Wingate-based studies, it remains unknown whether an alteration in resistance during training provides a greater training benefit. Therefore, further studies are required to determine an optimal strategy to maximise adaptations to sprint interval training.

#### *6.4.4 Long-term training adaptations*

Whilst it has been consistently reported that high-intensity training ( $\geq 85\% \dot{V}O_{2\text{max}}$ ) is superior for improving cardiorespiratory performance compared to moderate-intensity training ( $\sim 60$  to  $65\% \dot{V}O_{2\text{max}}$ ) with the same or greater training volume (Helgerud et al. 2007; Gormley et al. 2008; Matsuo et al. 2014), this might be simply due to the fact that the intervention duration (6 to 8 weeks) employed in these studies was too short to detect significant improvements in the moderate-intensity training. Gaesser and Rich (1984)

reported the time course of changes in  $\dot{V}O_{2\max}$  in response to high-intensity (80 to 85%  $\dot{V}O_{2\max}$ , HI group) and low-intensity (45%  $\dot{V}O_{2\max}$ , LO group) exercise training over 18 weeks. Whilst greater increases in  $\dot{V}O_{2\max}$  were achieved in HI group (11.6%) compared to LO group (5.3%) during the first 6 weeks, a similar magnitude of changes in  $\dot{V}O_{2\max}$  was observed between the groups (HI vs. LO: 15.7 vs. 12.7%) at 18 weeks. Similarly, Astorino et al. (2013) saw greater gains in  $\dot{V}O_{2\max}$  in high-intensity interval training (6 to 10 x 60-s cycling at 80 to 90%  $\dot{V}O_{2\max}$  interspersed with 75-s recovery) compared to moderate-intensity interval training (the same training protocol but performed at 60 to 80 %  $\dot{V}O_{2\max}$ ) during the first 3 weeks; however, overall improvement at 12 weeks was not different between the groups. This may suggest that although exercise intensity has an impact on the time course of changes in  $\dot{V}O_{2\max}$ , similar overall improvement is achieved regardless of intensity, provided that the minimum intensity for improving  $\dot{V}O_{2\max}$  is ensured (Swain and Franklin 2002).

Using Sprague-Dawley rats, it has been demonstrated that while high-intensity exercise training at 85 to 90%  $\dot{V}O_{2\max}$  results in myocardial adaptations (e.g. hypertrophy, an increased contractile capacity) within a few weeks, such effects reach a plateau at approximately 6 to 8 weeks (Wisløff et al. 2001; Kemi et al. 2004, 2005). This is in line with the study by Astorino et al. (2013) who observed a plateau in  $\dot{V}O_{2\max}$  gains in the high-intensity training group after 6 weeks. On the other hand, there was a trend for the moderate-intensity training group to increase  $\dot{V}O_{2\max}$  towards the end of the study (12 weeks) (Astorino et al. 2013). Nevertheless, the moderate-intensity group performed at higher intensity during the second 6 weeks (70 to 80%  $\dot{V}O_{2\max}$ ) of the study compared to the first 6 weeks (60 to 75%), whereas the relative intensity in the high-intensity group was kept constant throughout 12 weeks (80 to 90%  $\dot{V}O_{2\max}$ ) (Astorino et al. 2013). Furthermore, although Gaesser and Rich (1984) increased total exercise volume from the first training week to the last week (from 300 to 350 kcal per session) in both high-intensity and low-intensity training groups, relative

exercise intensity was remained unchanged throughout 18 weeks, and both groups showed a levelling-off of  $\dot{V}O_{2\max}$  improvements by 15 weeks (Gaesser and Rich 1984). This indicates that exercise intensity rather than volume may play a role in continuous cardiorespiratory improvements. Scharhag-Rosenberger et al. (2009) investigated effects of exercise training at 60% heart rate reserve (HRR) over 1 year and showed that the change in  $\dot{V}O_{2\max}$  reached a plateau after 6 months. However, neither exercise intensity nor volume was altered over the year, and further improvements might have been seen with alterations in exercise stimulus (Scharhag-Rosenberger et al. 2009). Moreover, in the majority of previous Wingate-based studies, training frequency was set to be three days per week (Burgomaster et al. 2005, 2006, 2007, 2008; Gibala et al. 2006, Hazell et al. 2010; Macpherson et al. 2011; Trilk et al. 2011; Zelt et al. 2014); however, an optimal or minimum dose of HIT to promote training adaptations has yet to be determined. Early studies demonstrated that improvements in  $\dot{V}O_{2\max}$  were independent of training frequency (2 or 4 days $\cdot$ wk $^{-1}$ ) over 7 to 13 weeks in healthy men (Fox et al. 1975) and in women (Lesmes et al. 1978) using supramaximal-intensity interval training at 130 to 170%  $\dot{V}O_{2\max}$ . Nevertheless, submaximal HR was decreased to a greater extent when the interval training was performed more frequently (4 days $\cdot$ wk $^{-1}$ ) over longer duration (13 weeks), suggesting that increased training frequency or prolonged training period might provide greater improvements in autonomic nervous system (e.g. a decrease in sympathetic drive) or stroke volume (Fox et al. 1975; Billat 2001b). Further studies are required to determine an optimal combination of training intensity, volume and frequency as well as the time course of training adaptations in response to exercise training over an extended period ( $\geq 6$  months).

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